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Abstracts of Communications or Demonstrations must state the type of animal used (common name or genus, including man. Where applicable, abstracts must specify the anaesthetics used, and their doses and route of administration, for all experimental procedures (including preparative surgery, e.g. ovariectomy, decerebration, etc.).

For experiments involving neuromuscular blockade, the abstract must give the type and dose, plus the methods used to monitor the adequacy of anaesthesia during blockade (or refer to a paper with these details). For the preparation of isolated tissues, including primary cultures and brain slices, the method of killing (e.g. terminal anaesthesia) is required only if scientifically relevant. In experiments where genes are expressed in *Xenopus* oocytes, full details of the oocyte collection are not necessary. All procedures on human subjects or human tissue must accord with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission; authors must tick the appropriate box to indicate compliance.

SA01

The Physiological Society and the development of Physiology in Ireland, 1876–2026

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The 150th anniversary of The Physiological Society provides an opportunity to reflect both on the development of physiology as a discipline in Ireland and on the Society's sustained and continuing contribution to that development. In the late nineteenth and early twentieth centuries, the teaching of physiology in Irish universities was largely embedded within medical education and training. Over the course of the twentieth century, however, physiology increasingly developed as a distinct academic discipline and experimental science. This evolution was accompanied by the establishment and expansion of undergraduate degree programmes in physiology, which have educated and trained generations of physiologists and contributed to the development of physiological research across Ireland.

Irish physiology was central to the very genesis of the Society. Gerald Francis Yeo, a graduate of Trinity College Dublin who studied and taught physiology in Dublin before his appointment as Professor of Physiology at King's College London, was a founding member and served as secretary from 1876 until 1889. Engagement with the Society has contributed to the growth, diversification and internationalisation of Irish physiology. The Society's scientific meetings held in Ireland provide a particularly valuable record of its engagement with, and influence on, research in this country. The first documented meeting of the Society in Ireland took place in Dublin in 1948, hosted jointly by Trinity College Dublin, University College Dublin and the Royal College of Surgeons in Ireland [1]. This was followed by meetings at University College Galway in 1965 and University College Cork in 1973, bringing the Society into direct contact with an increasingly established physiological research community outside Dublin. Further meetings were held in Dublin, Cork, Belfast and Dundalk throughout the late 20th and early 21st centuries, reflecting both the geographical spread and growing diversity of physiological research across the island.

The Society's journals have provided an equally important platform for the dissemination of physiological research from Irish universities. Numerous publications by researchers based in Ireland have appeared in *The Journal of Physiology* and *Experimental Physiology*, while Irish academics have also contributed to the leadership of these journals through senior editorial roles. Through these activities, the Society has provided not only a forum through which research from Ireland could be communicated to an international audience, but also a channel through which developments in international physiology could influence research, teaching and professional development within Ireland.

SA02

UCD Physiology: 1854 to the present day

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The Catholic University of Ireland was founded in 1854, becoming University College Dublin (UCD) in 1908. John Henry Newman was appointed the first Rector of the University and one year later established the Catholic University Medical School in Cecilia Street. He nominated Robert Dyer Lyons as Professor of Physiology in 1855 thus beginning the long history of Physiology in UCD. Over the intervening years, UCD Physiology has made major contributions to education and research in the University and its graduates and staff have gone on to play leading roles nationally and internationally, including the active parts taken by UCD Physiologists in the Physiological Society. Amongst the most famous of these was EJ Conway FRS who completed a BSc and MSc in Physiology in the School of Medicine before returning to his medical studies. He then lectured in UCD Physiology, was later appointed first Professor Biochemistry in the University and made major advances in the elucidation of the ionic basis of the membrane potential, the understanding of the regulation of cell volume and the role of cellular respiration in acid production and secretion.

The Department of Physiology later moved to Earlsfort Terrace, then the University's main campus, and subsequently to its current home in Belfield. With fitting symmetry, many of the current staff in Physiology are members of the Conway Institute of Biomolecular and Biomedical Research, a multi-disciplinary research Institute, named after one of its most famous graduates. This provides an environment in which Physiological research can take advantage of the power of the latest omics, bioinformatic and imaging advances to solving key questions in biomedical research. Our medical students, our BSc Physiology graduates and our post research graduates go on to successful careers in science, medicine, education and other areas making major contributions in their chosen fields.

SA03

Development and Feasibility of a Remotely Delivered Home-Based Physical Activity and Exercise Intervention for Adults with Pulmonary Hypertension.

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Pulmonary hypertension (PH) is associated with a substantial symptom burden, reduced exercise capacity and impaired quality of life (QoL). Although exercise is recommended as an adjunct to medical therapy, physical activity (PA) levels remain low and access to specialist, centre-based programmes is limited throughout Europe. This study aimed to develop and evaluate a patient-centred, theoretically informed, remotely delivered home-based PA and exercise intervention for adults with PH. Following Medical Research Council guidance for developing complex interventions, virtual semi-structured interviews and thematic analysis was used to explore the attitudes, knowledge, preferences and support needs of 19 adults with PH. Fear of overexertion, physical harm and breathlessness strongly constrained physical activity. Participants reported receiving inadequate exercise guidance and expressed a preference for individualised home-based exercise supported by clinicians, monitoring and accountability. These qualitative findings informed a single-arm feasibility study involving 20 clinically stable adults with PH (80% female; age 49.9 ± 15.9 years). Participants completed a 10-week remotely delivered programme comprising induction sessions, education, aerobic, resistance and respiratory training, a PA manual and logbook, wearable activity monitoring and health coaching. Feasibility, safety, acceptability, PA, functional capacity, QoL and self-efficacy were assessed; 19 participants completed follow-up. No intervention-related adverse events occurred. Adherence to prescribed aerobic, resistance and respiratory training was 95%, 91% and 94%, respectively. Six-minute walk distance increased by 72 m ($p < 0.001$), daily steps by 2,349 ($p < 0.001$), and moderate-to-vigorous PA from 9.3 to 23.6 min/day ($p < 0.001$). Sedentary waking time decreased, while lower-limb function, PH-specific QoL and most self-efficacy domains improved. Participants valued the programme's convenience, flexibility, accessibility and professional support. This fully remote, home-based intervention was safe, feasible and acceptable, with promising improvements in PA, functional capacity and QoL. It may help address important barriers to centre-based programme participation. A multicentre randomised controlled trial is warranted to establish clinical effectiveness and long-term sustainability.

SA04

Studying the clots that cause strokes

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Stroke is a leading cause of death and disability worldwide. Over 1.1 million people in the EU and 15 million people worldwide suffer a stroke each year. The majority (80-85%) of strokes are acute ischemic strokes, caused by blockage of a cerebral vessel by a thrombus or embolus. The mortality is as high as 40% and over half of those who survive are left with life changing disability. Approval of a thrombolytic drug (tissue plasminogen activator (tPA)) to dissolve stroke-causing blood clots in the late 1990's led to the first major clinical development in the treatment of acute ischemic stroke. In the last ten years, an endovascular surgical procedure called mechanical thrombectomy has radically changed stroke treatment, quickly becoming a gold-standard intervention to remove the clot at specialist stroke centres.

Not all clots that cause strokes are the same. The composition of blood clots can vary considerably and significantly influences the outcome for patients treated with thrombolytic and/or mechanical thrombectomy devices. In collaboration with partners in stroke centres internationally, my research team have developed two of the largest registries of stroke clots in the world, called the RESTORE registry and the CLOTBASE registry. Through our work studying the clots that cause strokes, we aim to improve acute ischemic stroke treatment by advancing medical device design to remove stroke-causing clots optimally. Additionally, our work aims to discover diagnostic biomarkers that can lead to improved diagnostic tests in the emergency care setting, new therapeutic strategies to dissolve clots safely and to prevent strokes in the future. This talk will give some examples of advancements we have made in this area.

SA05

Using new tools to answer old questions: Insights from urethral smooth muscle physiology

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Urethral smooth muscle (USM) contracts to generate myogenic tone during bladder filling, thus maintaining urinary continence by preventing urine leakage. Historically, USM physiological investigation has largely utilized mammalian models such as pigs, guinea-pigs and rabbits, which have provided a wealth of information on how different cell types and ionic conductances contribute to USM tone and function. For example, urethral tone was hypothesized to originate from Ca^{2+} influx into USM cells (USMC) via a window current for voltage-dependent L-type Ca^{2+} channels, as drugs such as nifedipine reduced tone in these species. Previous studies from our laboratory identified a population of spontaneously active, non-contractile cells in rabbit USM termed interstitial cells of Cajal-like cells (ICC-LC) which we purported to serve as electrical pacemakers to USMC to control tone (Sergeant *et al.*, 2000). Rabbit ICC-LC exclusively expressed the Ca^{2+} -activated- Cl^- channel ANO1, and inhibitors of Ca^{2+} -activated- Cl^- channels drastically reduced rabbit urethral tone and contractions evoked by neurotransmitters (Fedigan *et al.*, 2017).

More recently, our laboratory has sought to further elucidate the cell types and ionic conductances that regulate USM contractility using transgenic mouse models, combined with the most up to date pharmacological targeting of ion channels suggested to play a role in USM contractility. Using a genetically encoded Ca^{2+} reporter expressed exclusively in either USMC or ICC-LC, we have been able to characterize the underlying Ca^{2+} signals in USM that generate myogenic tone. Asynchronous Ca^{2+} waves in USMC bundles cause uncoordinated contractions across the sphincter, which summate to generate uniform tone (Drumm *et al.*, 2018). Cell-specific optogenetic reporter mice have allowed us to unambiguously identify and stimulate ICC-LC within intact tissues, allowing us to report, to our surprise, that while ICC-LC are a dynamically active and distinct cell type in the urethra, they do not appear to act as pacemakers nor neuromodulators in mice (Gupta *et al.*, 2024). Furthermore, using our in-situ Ca^{2+} imaging capacity along with potent and specific modulators of ANO1 and L-type Ca^{2+} channels, we have again to our surprise found that neither conductance is required for normal USM Ca^{2+} activity nor contractility, but instead that store-operated Ca^{2+} entry is an essential pathway required for normal USM function (Gupta *et al.*, 2025).

Our research at Dundalk Institute of Technology highlights how the advent of nascent technologies and tools allows us to re-examine conclusions drawn from previous studies and to further advance our understanding of complex physiological processes across different species.

C01

The Role of Kv7 Channels in Regulating Neurogenic Contractions and Relaxations in the Corpus Cavernosum

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Erectile dysfunction (ED) is characterised by impaired relaxation of corpus cavernosum smooth muscle (CCSM), leading to reduced penile blood filling and failure to achieve or maintain erection. Although phosphodiesterase-5 inhibitors improve erectile function by enhancing nitric oxide (NO)-mediated signalling, many patients, particularly those with diabetes or vascular disease, exhibit a poor therapeutic response. Voltage-gated potassium (Kv7) channels regulate membrane potential and smooth muscle excitability and may represent an alternative therapeutic target. This study investigated the role of Kv7 channels in regulating neurogenic contractile and relaxation responses in mouse CCSM.

Corpus cavernosum strips were isolated from adult male C57BL/6 mice and mounted in organ baths for isometric tension recordings. Electrical field stimulation (EFS; 0.5–8 Hz, 30 s, 0.3 ms pulse width) was used to evoke neurogenic responses. Contractile responses were examined in the presence of the nitric oxide synthase inhibitor N ω -nitro-L-arginine (L-NNA), while relaxation responses were assessed following pre-contraction with phenylephrine and guanethidine. Kv7 channels were activated using ML213 (5 μ M) and inhibited using linopirdine (10 μ M) or XE991 (10 μ M). The contribution of large-conductance Ca²⁺-activated potassium (BK) channels was investigated using Penitrem A (300 nM). Data were analysed using two-way ANOVA followed by Šídák's multiple comparisons test. All animal procedures were approved by the Dundalk Institute of Technology Animal Use and Care Committee and conducted in accordance with EU Directive 2010/63/EU.

EFS evoked frequency-dependent contractions that increased with stimulation frequency. Activation of Kv7 channels with ML213 significantly reduced contraction amplitude in preparations (n=3) at 0.5 Hz (P = 0.0141), 1 Hz (P = 0.0008), 2 Hz (P = 0.0005), 4 Hz (P = 0.0006), and 8 Hz (P = 0.0006), with a corresponding reduction in contractile area across all frequencies. Inhibition of Kv7 channels with linopirdine significantly increased relaxation amplitude at 1 Hz (P = 0.0038), 2 Hz (P = 0.0008), 4 Hz (P = 0.0009), and 8 Hz (P = 0.0048; n = 3), although relaxation area was not significantly altered. Sequential inhibition of BK channels with Penitrem A following Kv7 blockade (n = 2) produced no further enhancement of relaxation responses, indicating a minimal contribution of BK channels under these experimental conditions.

These findings demonstrate that Kv7 channels play a key role in regulating neurogenic contractile activity in mouse CCSM by limiting membrane excitability and phasic contraction. The increased relaxation observed following Kv7 inhibition is likely secondary to enhanced pre-stimulation contractile tone rather than direct modulation of nitrergic signalling. In contrast, BK channels appear to contribute minimally to EFS-evoked relaxation under these conditions. Pharmacological modulation of Kv7 channels may therefore represent a promising therapeutic strategy for ED, particularly in patients with impaired NO-dependent relaxation. Taking all together, these findings raise the possibility that NO-dependent neurogenic relaxations may be largely independent of Kv7-mediated membrane potential regulation, suggesting that membrane potential primarily regulates contractile rather than nitrergic relaxation mechanisms.

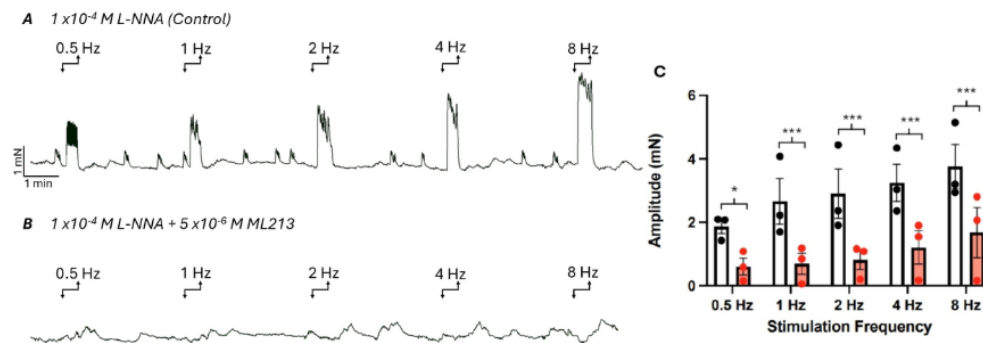


Figure 1. Effect of Kv7 Channel Activation (ML213) on EFS-Evoked Contractions in Mouse Corpus Cavernosum

C02

Acute Effects of High-Intensity Interval Exercise versus Moderate-Intensity Continuous Exercise on Dynamic Cerebral Autoregulation in Inactive Adults

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Background: Dynamic cerebral autoregulation (dCA) maintains stable cerebral blood flow despite transient fluctuations in arterial blood pressure. While acute exercise is a potent stimulus for cerebrovascular adaptation, it also imposes substantial haemodynamic stress on the cerebral circulation. High-intensity interval exercise (HIIE) elicits cardiovascular and metabolic adaptations comparable to, or greater than, those achieved with moderate-intensity continuous exercise (MICE); however, its acute effects on cerebrovascular regulation remain poorly understood. Therefore, this study compared the acute effects of HIIE and MICE on dCA in inactive adults.

Methods: Twelve inactive adults (5 females, age: 50.3 ± 9.3 years, BMI: 27.2 ± 4.7 kg/m², $\dot{V}O_{2\max}$: 27.9 ± 9.3 mL/kg/min) completed four laboratory visits. During the initial visit, participants performed a ramp incremental cycling test to volitional exhaustion to determine maximal oxygen uptake ($\dot{V}O_{2\max}$) and establish individualised exercise intensities. Participants subsequently completed three randomised, time-matched (30 min) experimental conditions: moderate-intensity continuous exercise (MICE; 90% gas exchange threshold), high-intensity interval exercise (HIIE; 10 x 1-min intervals at 70% Δ), and a seated control condition (CON). dCA was assessed before, and 10 and 60 min following each condition using a 5-min repeated sit-to-stand manoeuvre performed at 0.05 Hz. Middle cerebral artery blood velocity (MCAv) and arterial blood pressure (ABP) were continuously measured using transcranial Doppler ultrasonography and finger photoplethysmography, respectively. dCA was quantified using transfer function analysis (TFA), from which gain, normalised gain, phase, and coherence were determined, and subsequently analysed using a two-way repeated-measures ANOVA (time x condition).

Results: No significant main effects of condition or time, nor condition x time interactions, were observed for TFA gain (all $P \geq 0.40$, $\eta^2 \leq 0.08$), normalised gain (all $P \geq 0.58$, $\eta^2 \leq 0.045$), phase (all $P \geq 0.27$, $\eta^2 \leq 0.11$), or coherence (all $P \geq 0.31$, $\eta^2 \leq 0.10$).

Conclusion: This study provides novel evidence that a single bout of HIIE and MICE do not alter dCA in inactive adults, suggesting that cerebral pressure-flow regulation is preserved despite the challenging haemodynamic demands of HIIE.

C03

Cardiorespiratory fitness and sex impact the colorectal cancer-suppressive effects of aerobic exercise serum

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Introduction and aims

Post-exercise human serum treatment can exhibit anti-viability properties in various cancer cell lines. However, whether the presence and/or magnitude of the effect differs in different participant populations is yet to be established. The aim of this study was to characterise baseline differences in serum-stimulated colorectal cancer cell viability and the acute exercise-induced response in males and females with varying levels of cardiorespiratory fitness (CRF).

Methods

Forty-one participants (13 female) were stratified by maximal exercise test-derived VO₂peak into high ($n = 20$) and low CRF ($n = 21$) and completed two experimental trials in a randomised crossover design: (1) 30-min moderate-intensity aerobic exercise and (2) 30-min seated rest. Serum samples were collected before (pre), immediately after (post0), and 60-min after (post60) each trial and used to treat the human colorectal cancer cell line Caco-2 for 48 h, with cell viability assessed by MTT assay. Data were analysed using linear mixed-effects models.

Results

Exercise-induced alterations in Caco-2 cell viability were, at least in part, modulated by CRF and sex. Specifically, exercise serum significantly reduced cell viability compared with no-exercise control serum when derived from individuals with high (post0: -6.26%, $P = 0.027$; post60: -8.86%, $P = 0.010$) but not low CRF (post0: -3.19%, $P = 0.21$; post60: -7.71%, $P = 0.085$), and when derived from males (post0: -5.59%, $P = 0.014$; post60: -10.41%, $P = 0.001$) but not females (post0: -2.68%, $P = 0.39$; post60: -3.46%, $P = 0.63$). At rest, there were no CRF ($P = 0.78$) or sex differences ($P = 0.90$) in the effects of basal human serum on Caco-2 cell viability.

Conclusion

Acute aerobic exercise-induced changes in the circulating milieu can have suppressive effects on colorectal cancer cell viability in vitro, which may be CRF- and sex-dependent. These findings provide the first insights into the role of individual characteristics in determining the effects of exercise on colorectal

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cancer cells, with potential implications for increasing the precision of exercise recommendations for colorectal cancer risk reduction.

C04

Differential remodelling of intracellular Ca^{2+} homeostasis by clinically relevant triple-negative breast cancer (TNBC)-associated TP53 variants: implications for mutation-directed therapy

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Introduction: Triple-negative breast cancer (TNBC) accounts for 10–15% of breast cancers and lacks established hormone- or HER2-directed therapies, contributing to poor clinical outcomes. Up to 80% of TNBCs harbour *TP53* mutations. Beyond its canonical transcriptional functions, p53 regulates apoptosis through transcription-independent control of endoplasmic reticulum (ER)-to-mitochondrial Ca^{2+} signalling. Our previous multidimensional profiling of an isogenic MCF10A TP53-variant panel identified mutation-specific heterogeneity in apoptosis resistance, migration, invasion and anoikis. Whether this functional heterogeneity is accompanied by distinct Ca^{2+} -signalling phenotypes that influence mitochondrial function and apoptotic sensitivity remains unclear.

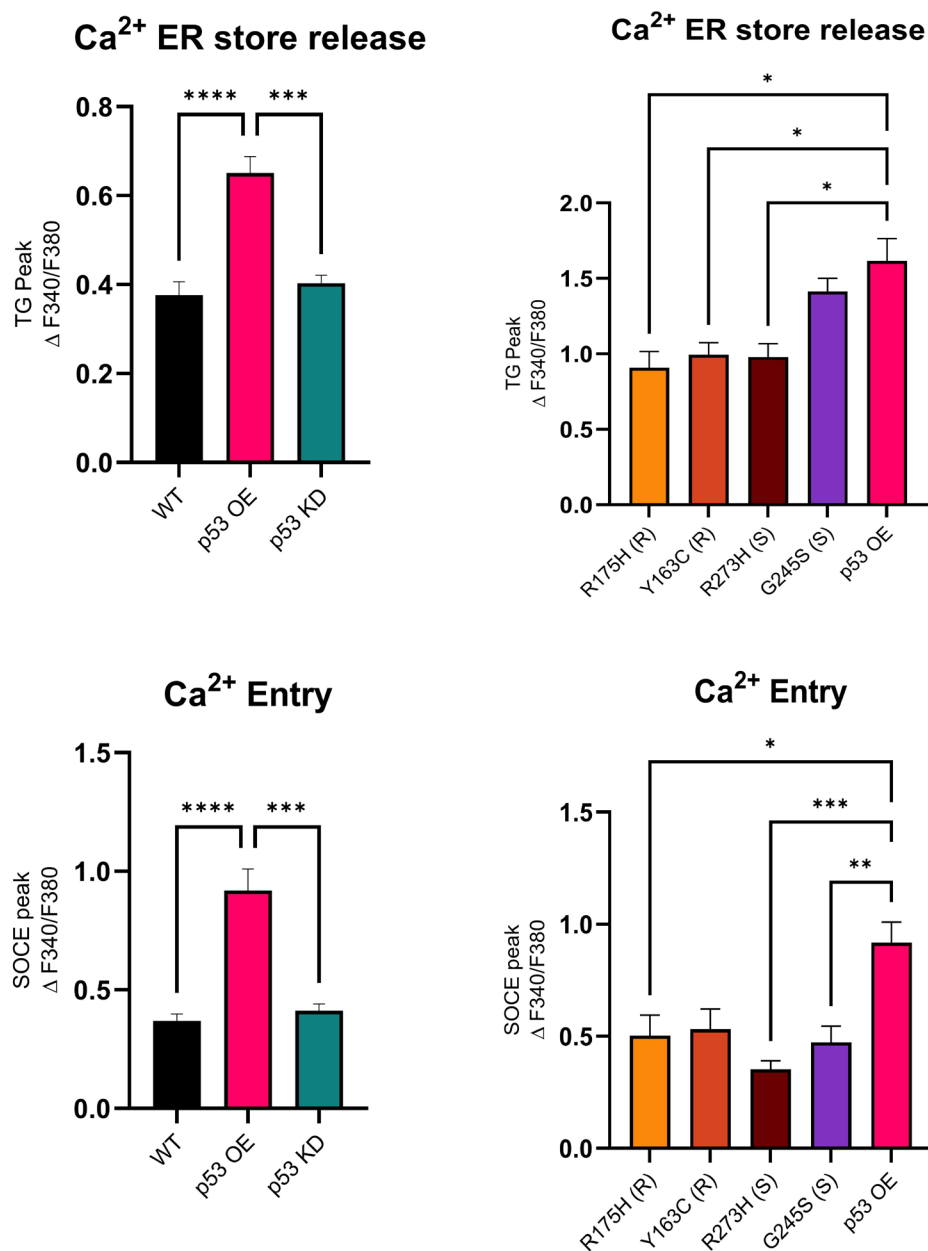
Aim: To determine how *TP53* mutation status alters ER Ca^{2+} release, store-operated Ca^{2+} entry (SOCE) and mitochondrial bioenergetics using an isogenic panel of p53-altered MCF10A mammary epithelial cells.

Methods: Isogenic MCF10A cells expressing endogenous wild-type p53 (WT), p53 knockdown (KD), wild-type p53 overexpression (p53-OE), or clinically relevant *TP53* missense variants (R175H, Y163C, G245S and R273H) were studied. Intracellular Ca^{2+} was measured using Fura-2-AM during thapsigargin-induced ER Ca^{2+} depletion, followed by extracellular CaCl_2 addition to quantify SOCE. Calcium experiments comprised four independent biological replicates ($n=4/\text{group}$) and were analysed using Kruskal–Wallis tests with Dunn’s post hoc correction. Mitochondrial bioenergetics were assessed using the Agilent Seahorse XFp Mito Stress Test (preliminary; $n=1$). No human participants, human tissue or animals were involved.

Results: p53-OE significantly increased ER Ca^{2+} release compared with WT and KD ($p<0.0001$ and $p=0.0003$, respectively) and increased SOCE compared with WT and KD ($p<0.0001$ and $p=0.0006$). WT and KD responses were indistinguishable ($p>0.9999$), consistent with the low basal abundance of p53 in MCF10A cells. Consequently, p53-OE enabled resolution of a p53-dependent Ca^{2+} phenotype against which *TP53* variants could be compared. Relative to p53-OE, ER Ca^{2+} release was reduced in R175H ($p<0.01$), R273H ($p<0.05$) and Y163C ($p<0.05$), whereas G245S was unchanged. SOCE was reduced in R175H ($p=0.0312$), R273H ($p=0.0001$) and G245S ($p=0.0062$), whereas Y163C was unchanged. Alongside our previous phenotypic profiling (Pal et al, 2023, NPJ Breast Cancer), G245S and Y163C retained different components of the p53-OE Ca^{2+} phenotype and displayed contrasting apoptotic and migratory behaviours: G245S retained ER Ca^{2+} release and had shown greater sensitivity to doxorubicin-induced apoptosis with lower migratory activity, whereas Y163C retained SOCE and displayed relative apoptosis resistance with higher migratory activity. Preliminary Seahorse analysis demonstrated reduced basal respiration and spare respiratory capacity in p53-OE, suggesting that enhanced ER Ca^{2+} mobilisation may

be accompanied by reduced mitochondrial oxidative metabolism and bioenergetic reserve. These findings require confirmation in additional biological replicates.

Conclusion: Wild-type p53 overexpression revealed a calcium-regulatory phenotype characterised by enhanced ER Ca^{2+} mobilisation and SOCE. Clinically relevant TP53 mutations remodelled this phenotype in a mutation-specific manner, generating distinct Ca^{2+} -signalling profiles alongside heterogeneous tumour-associated behaviours. These findings establish TP53 mutation status as an important determinant of intracellular Ca^{2+} homeostasis and provide a physiological framework for studies of ER-to-mitochondrial Ca^{2+} transfer, mitochondrial metabolism and apoptotic sensitivity, with the aim of informing mutation-directed therapeutic strategies in TNBC.



C05

Astrocytes display regional differences in their physiological responses to pro-inflammatory stimulation

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Introduction: Astrocytes play integral roles in maintaining homeostasis and surveying the micro-environment in the healthy CNS. Under pathological conditions, such as Alzheimer's and Parkinson's disease, astrocytes become activated and contribute to disease progression. The reactive astrocyte phenotype arises from a range of pathological scenarios where astrogliosis leads to further pro-inflammatory cytokine production and thus, neuronal damage and death. Although astrocytes are historically viewed as a uniform glial population, in recent years they have been recognised as highly diverse at molecular, morphological and functional levels. Transcriptomic and proteomic studies revealed pronounced astrocyte heterogeneity across brain regions, suggesting that this heterogeneity is shaped by developmental patterning cues, intra- and extracellular signals and neuronal interactions. Induced pluripotent stem cell (iPSC)-derived astrocytes provide an excellent system for studying region-specific astrocytes. When guided by appropriate patterning cues, iPSC can be differentiated into neural progenitors with forebrain, midbrain, hindbrain or spinal identities, which then can be further differentiated into regionally distinct astrocyte progenitors.

Aims: Our research has focused on cortical and ventral midbrain differentiation in line with our interest in Alzheimer's disease and Parkinson's disease. It is the aim of this work to directly compare cortical (CTX) and ventral midbrain (VM) astrocytes from the same iPSC line to identify differences in morphology, gene expression, protein level and functionality.

Methods: For this we have differentiated the same parental iPSC line into cortical and ventral midbrain neural astrocytes. With these astrocytes we explored changes across morphology (ICC), gene and protein expression (qPCR, ELISA, Bulk RNA sequencing) and functionality (Seahorse and calcium imaging) at baseline and in response to inflammatory stimulation.

Results: We have observed clear morphological, transcriptomic and functional differences in these regional iPSC-derived astrocytes both at baseline and in response to pro-inflammatory stimulus (n=3 differentiations into astrocytes from iPSCs, with three technical replicates per experiment). This indicates that these cells may play very different roles in the different regions of the brain and highlighting the critical importance of selecting the appropriate region for the study being conducted.

C06

Modelling epilepsy-associated overexpression of miR-134 produces single cell and network functional and structural changes in the hippocampus of naïve mice

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MicroRNA-134 (miR-134) is a brain-enriched noncoding RNA upregulated in temporal lobe epilepsy that regulates the levels of proteins controlling dendritic structure, ion channel expression, and neuronal excitability. We recently demonstrated that miR-134 inhibition using antisense oligonucleotides (antimir-134) reduces spontaneous recurrent seizures (SRS) in epileptic male mice, also attenuating CA1 neuronal epilepsy hallmarks such as intrinsic hyperexcitability, exacerbated synaptic transmission and impaired long-term potentiation (LTP). It remains unproven, however, if increased miR-134 levels alone are sufficient to produce hyperexcitability or epilepsy-associated co-morbidities.

We combined electrophysiological, morphological and behavioral analyses to investigate the effects of increased miR-134 modelled to a level that matches human TLE. Viral vector-directed miR-134 overexpression was achieved in the hippocampi of naïve mice of both sexes, followed by behavioral testing, patch-clamp experiments to principal hippocampal neurons and post-hoc morphological analyses of biocytin-filled CA1 neurons. We also used recordings of local field activity to investigate evoked epileptiform-like activity susceptibility, synaptic strength and plasticity magnitude, and probability of excitatory neurotransmitter release.

Overexpression of miR-134 in naïve mice (AAV.miR134) (+4.85±0.8-fold increase, n=9 mice/group, p=0.015 Welch's test) to mimic findings in human epilepsy resulted in sex-specific effects on naturalistic behavior, consistent with enhanced exploratory or risk-tolerant behavior. AAV.miR134 was sufficient to increase cell excitability of transduced CA1 pyramidal neurons (n=10 mice/group, p<0.001, LMM), associated with an alteration of action potential afterdepolarization slope (n=10 mice/group, p=0.036, LMM) and delayed voltage-gated potassium peak activation (n=10 mice/group, p=0.04, LMM). Furthermore, morphological analyses of patched CA1 neurons indicated selective reduction of apical (n=10 mice/group, p=0.006, Mann-Whitney), but not basal, dendritic complexity and spine density (n=10 mice/group, p=0.026, unpaired t). This suggests a structural substrate for altered network integration and neuronal excitability[PQ1]. Functionally, synaptic strength, measured as local field response to electrical stimuli, was enhanced in both major CA1 strata in AAV.miR134 animals (n=6 mice/group, p<0.05, 2way ANOVA interaction), whereas neurotransmitter release was selectively enhanced at SC-CA1 synapse (n=8 mice/group, p=0.043, Mann-Whitney), indicating circuit-specific presynaptic modulation. Despite increased synaptic strength and NT release in SC-CA1, TBS-induced LTP magnitude was comparable to controls. In contrast to controls, post-LTP NT release was not further increased, consistent with a reduced dynamic range of presynaptic plasticity. Despite observed CA1 cellular and circuit altered phenotypes, epileptiform-like activity evoked by modified artificial cerebro-spinal fluid (maCSF, 0.25 mM Mg²⁺, 8mM K⁺) perfusion trended increased power in area CA3 (n=3 animals/group, p=0.058, Mann-Whitney), but not CA1, on AAV.miR134 slices in an interface chamber setup.

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Our findings showcase that miR-134 is a critical regulator of neuronal excitability, dendritic architecture and population synchronization in the hippocampus, and suggest a role of miR-134 overexpression alone to increase seizure susceptibility in *ex vivo* slices. Future studies should investigate whether miR-134 overexpression is sufficient to manifest *in vivo* spontaneous seizures, alone or in combination with a second hit. Altogether, our results strengthen the rationale for targeting miR-134 as a disease-modifying therapeutic strategy in epilepsy and provide new mechanistic insight into how microRNAs shape neuronal and circuit-level function.

C07

Rilmenidine prevents sympathetic and cardiovascular alterations in a rat model of insular hemorrhagic stroke

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Introduction Insular stroke results in marked cardiovascular changes, and the mechanisms need to be investigated. We have recently developed a murine model of hemorrhagic insular stroke that can help us understand such mechanisms [Fontes MAP et al. (2024). Clin Auton Res 34(6), 549-560; Marins FR et al. (2020). Auton Neurosci 227, 102695]. Rilmenidine, a centrally acting antihypertensive drug, is known to reduce sympathetic activity. Previous evidence suggests that rilmenidine effects result from activation of imidazoline receptors located in premotor sympathetic neurons at the rostral ventrolateral medulla. Thus, exploring the effects of rilmenidine in rats subjected to insular stroke may help: 1) to confirm that insular stroke generates a central abnormal sympathetic tone and 2) to identify benefits of rilmenidine that may potentially attenuate post-insular stroke cardiovascular alterations.

Aims This study evaluated the effects of rilmenidine on cardiovascular, neurobehavioral and cardiac norepinephrine levels in our newly developed rat model of insular hemorrhagic stroke (ICH).

Methods Ethics approval CEUA UFMG 109/2022. Male Wistar rats were anesthetized, instrumented for injection into the insular cortex of autologous blood (ICH, 200 nL) or vehicle (saline 0.9%, IC-Sal, 200 nL; central volume control) and prepared for recording of mean arterial pressure (MAP) and heart rate (HR). Three groups (n=8-9 each) were subjected to three days of intraperitoneal treatment as follows; 1) IC-Sal treated with vehicle (NaCl 0.9%, 0.1ml /100g); 2) ICH treated with vehicle (ICH-Sal, NaCl 0.9%, 0.1ml /100g); 3) ICH treated with rilmenidine (ICH-Ril 10 µg/kg). Anxiety, activity and muscle strength were evaluated. Cardiac norepinephrine was quantified by HPLC. One-way ANOVA was used for statistical analysis.

Results ICH-Sal rats exhibited higher baseline MAP (IC-Sal: 104±4 mmHg vs. ICH-Sal: 120±14 mmHg; p<0.01) and HR (IC-Sal: 314±21 bpm vs. ICH-Sal: 460±41 bpm; p<0.01). Rilmenidine completely prevented the ICH-evoked increases in MAP and HR (ICH-Ril: MAP: 103±5 mmHg; HR: 295±22 bpm; p<0.01). No significant neurobehavioral changes were observed. ICH increased norepinephrine cardiac levels (IC-Sal: 17±9 ng.mg⁻¹ vs. ICH-Sal: 37±4 ng.mg⁻¹; p=0.0101), that were normalized with rilmenidine (ICH-Sal: 37±4 ng.mg⁻¹ vs. ICH-Ril: 7±7ng.mg⁻¹; p=0.0007).

Conclusions Our findings with rilmenidine suggest that insular stroke generates a centrally mediated abnormal sympathetic tone. Rilmenidine prevented the cardiovascular alterations resulting from insular hemorrhagic insular stroke via reduction of noradrenergic input to the heart. Our data indicates that

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rilmenidine may reduce post-stroke acute cardiovascular risk. (Support: FAPEMIG APQ-01128-21; CNPq MAPF PQ 301556/2026-1; ACVG 165774/2021-5; CNPq INCT 406792/2022-4).

C08

Evaluation of carotid body chemoafferent patterning in response to chronic hypoxia

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Introduction: Chronic Hypoxia (CH) is a key feature and a major driver of pathology in Chronic Obstructive Pulmonary Disease (COPD). Emerging evidence suggests that carotid body hyperactivity in CH has an important role in causing cardiovascular pathology. The carotid body inputs signals to the brainstem through chemoafferent fibres running in the carotid sinus nerve (CSN), a branch of the glossopharyngeal nerve. The traditional view is that a rise in mean chemoafferent frequency is the sole signal for reflex activation. However, no study has assessed a potential alteration in the pattern of chemoafferent firing, which may code for selective reflex activation and cardiovascular-respiratory adjustment.

Aims and Objectives: This study compared the baseline firing patterns of single carotid body chemoafferent fibres recorded from normoxic (N) and chronically hypoxic (CH) animals.

Method: Extracellular *ex vivo* recordings of chemoafferent activity were made from carotid bodies isolated from adult male Wistar rats (150-350g), following exposure to either 10 days of ambient air (N) or FiO₂ 12% (CH). Carotid bodies were isolated under terminal non-recovery anaesthesia (isoflurane 3-5% in O₂ 1.5 L min⁻¹), death by cervical dislocation and confirmed by exsanguination. Baseline activity was measured at a superfusate PO₂ of 300 mmHg, PCO₂ of 40 mmHg, and was captured for at least 100 seconds. Chemoafferent patterning was monitored by evaluating the variability of interspike intervals (ISI) and Shannon Entropy (the ability to predict the next ISI). Data presented as mean ± SEM. Statistical significance was taken as p<0.05; unpaired Student's t-test.

Results: A total of 80 single chemoafferent fibres were analysed (N, n=36) and (CH, n=44), and total spike counts were 3309 for N and 9463 for CH. The CH group exhibited a significant decrease in SD ISI (N: 3741 ± 401 vs. CH: 1071 ± 99 ms, p<0.0001) and co-efficient of variation (SD/Mean ISI) (N: 1.18 ± 0.029 vs CH: 1.06 ± 0.014 AU, p<0.0001). Poincaré plot analysis of ISI, illustrated a decrease in both short-term (SD1) (N: 5385 ± 603 vs. CH: 1517 ± 147 ms; p<0.0001) and long-term (SD2) (N= 7089 ± 782 vs. CH= 2168 ± 199 ms; p<0.0001) variability, and a decrease in overall ellipse area, consistent with a more regular firing pattern. The total Shannon Entropy was also decreased in the CH group (N: 1.572 ± 0.03 vs. CH: 1.390 ± 0.01 bits, p<0.0001), indicative of a more predictable spike pattern.

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Conclusions: In addition to increasing the mean frequency, CH exposure fundamentally reshapes the baseline carotid body chemoafferent discharge pattern. Key changes included a reduction in the variability and spread of ISIs, and an increase in predictability. This alteration in pattern may modify central signal integration thereby contributing to hypersensitive cardiovascular-respiratory reflex activation associated with CH.

C09

Transfer Function Analysis of Dynamic Cerebral Autoregulation in Individuals with Pre-Diabetes: A Pilot Study

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Dynamic cerebral autoregulation (dCA), the capacity of the cerebrovasculature to buffer transient fluctuations in arterial blood pressure, is critical for maintaining cerebral perfusion. Impaired dCA has been reported in individuals with type 2 diabetes, a population at elevated risk of cognitive decline. However, whether similar cerebrovascular dysfunction is evident in pre-diabetes (PD), an early stage of glycaemic dysregulation, remains unexplored. Furthermore, aerobic fitness is proposed to modulate cerebrovascular function, though its influence may be population-dependent.

Therefore, this pilot study used transfer function analysis (TFA), to assess whether (1) dCA is impaired in individuals with PD and (2) TFA-derived indices of dCA are associated with aerobic fitness.

Seven individuals with PD (5 females; 66 ± 6.6 years) and seven normoglycemic controls (CON) (4 females; 59 ± 3.55 years) volunteered to take part in this study, completing two visits. On visit one, participants performed a ramp incremental cycling test to exhaustion followed by a verification bout to determine maximal oxygen uptake ($\dot{V}O_{2\max}$). On visit two, dCA was assessed using a 5-minute repeated sit-to-stand manoeuvre completed at 0.05 Hz. Middle cerebral artery velocity (MCAV) and mean arterial pressure (MAP) were measured via Transcranial Doppler Ultrasonography and finger photoplethysmography respectively. dCA was quantified using TFA of mean MCAV and MAP, yielding values of gain, normalised gain, phase and coherence. Group differences were assessed using independent-sample t-tests, while Pearson correlations examined associations between $\dot{V}O_{2\max}$ and indices of dCA.

No between group differences were observed in gain ($P=0.91$), normalised gain ($P=0.15$), phase ($P=0.32$) or coherence ($P=0.07$). $\dot{V}O_{2\max}$ was not associated with gain, normalised gain, or phase in either group (all $p > 0.28$).

These preliminary findings suggest that dynamic cerebral autoregulation is preserved in pre-diabetes and is not associated with aerobic fitness. Although these findings suggest that early glycaemic dysregulation may not yet compromise dCA, larger studies are required to confirm these observations and to establish whether impairments develop with disease progression.

C10

Central arterial stiffness and cerebral pulsatility are not associated with cerebrovascular reactivity in inactive middle-aged adults

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Introduction and aim

Arterial stiffening increases the transmission of pulsatile energy into the cerebral circulation, and both arterial stiffness and cerebral pulsatility increase during middle age, potentially representing a critical window for cerebrovascular change. Carotid-femoral pulse wave velocity (PWV), a measure of central arterial stiffness is related to peripheral endothelial function, while middle cerebral artery (MCA) pulsatility index (PI) and the amplitude and kinetic cerebrovascular reactivity (CVR) responses to carbon dioxide are indicative of cerebrovascular function. Given PWV reaches the cerebral circulation through pulsatile transmission, and CVR is largely an endothelial-dependent response, central arterial stiffness could relate to cerebrovascular function through both pulsatile and endothelial pathways. However, evidence relating central arterial stiffness to CVR is limited. Given this, and that these relationships remain unexamined in inactive middle-aged adults, this study investigated whether PWV, PI, and the amplitude and kinetic CVR responses to hypercapnia are associated in this population.

Methods

Seventeen inactive middle-aged adults (10 males, 49.2±8.9 years) completed a single experimental visit. Carotid-femoral PWV was measured by applanation tonometry as the transit distance between the carotid and femoral sites divided by the pulse transit time, with ECG gating. Participants were then fitted with transcranial Doppler ultrasound and a gas analyser to continuously measure middle cerebral artery blood velocity (MCAv) and end-tidal CO₂ (PETCO₂). Hypercapnic CVR was assessed during 4 min of 6% CO₂ inhalation and the MCAv kinetic response to hypercapnia was modelled using a mono-exponential model to derive amplitude, time delay, time constant, and mean response time. Hypocapnic CVR was assessed during 1 min of voluntary hyperventilation at 25 breaths·min⁻¹. Resting PI was calculated as (MCAv maximum – MCAv minimum) / MCAv mean. Associations were assessed using partial correlations adjusting for age, with statistical significance set at $p < 0.05$.

Results

Hypercapnic CVR ($1.99 \pm 0.34 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$) was significantly associated with kinetic amplitude ($23.4 \pm 4.7 \text{ cm} \cdot \text{s}^{-1}$; $r = 0.63$, $p = 0.009$) but not with the time constant ($21.8 \pm 7.3 \text{ s}$), time delay ($15.8 \pm 4.1 \text{ s}$), or mean response time ($37.6 \pm 8.3 \text{ s}$; all $|r| \leq 0.23$, all $p \geq 0.395$). Hypocapnic CVR ($1.37 \pm 0.47 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$) was not associated with any kinetic parameter (all $|r| \leq 0.35$, all $p \geq 0.178$) or with hypercapnic CVR ($r = -0.26$, $p = 0.339$). Neither PWV ($7.6 \pm 1.7 \text{ m} \cdot \text{s}^{-1}$) nor PI (0.82 ± 0.10) were associated with hypercapnic CVR, hypocapnic CVR, or any kinetic parameter (all $|r| \leq 0.49$, all $p \geq 0.055$), and PWV and PI were not associated with each other ($r = -0.01$, $p = 0.985$).

Conclusion

Central arterial stiffness and MCA pulsatility were not associated with each other, nor with cerebrovascular reactivity or its kinetic parameters, in inactive middle-aged adults. This indicates that peripheral vascular measures may not reflect cerebrovascular function in this population. Further work

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in larger cohorts is needed to confirm these relationships and clarify the peripheral and cerebral vascular changes occurring in middle age.

C11

Acute effects of human versus digital voice coaching on maximal effort: a within-subject comparison across three high-effort physical tasks

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Abstract body

Introduction and aims: Verbal encouragement can enhance physical performance, but it remains unclear whether digitally delivered coaching can reproduce the effects of an in-person coach and whether these effects depend on the type of physical task. This study compared the acute effects of in-person human coaching, standardised digital voice coaching and an instructions-only control on physical performance, perceived exertion and motivation across three high-effort tasks.

Methods: Thirty-seven healthy adults (21 female, 16 male; aged 18–30 years) completed all three coaching conditions in a randomised within-subject design. Participants performed a wall-sit to volitional failure, a 60-s maximal handgrip task with and without visual feedback, and a 30-s Wingate cycling sprint. Performance, ratings of perceived exertion and task-specific motivation were assessed. Condition effects were analysed using Friedman tests with Bonferroni-corrected Wilcoxon signed-rank post-hoc comparisons. The study was approved by the Faculty of Health Sciences Research Ethics Committee, Trinity College Dublin (Ref. 251003), and all participants provided written informed consent.

Results: Wall-sit time-to-failure differed across conditions in females ($\chi^2(2)=18.13$, $p<0.001$, Kendall's $W=0.432$) and males ($\chi^2(2)=16.62$, $p<0.001$, $W=0.520$). Human coaching exceeded control in females ($p=0.004$), while both digital voice ($p=0.009$) and human coaching ($p=0.004$) exceeded control in males. Wingate peak relative power also differed across conditions in females ($\chi^2(2)=17.16$, $p<0.001$, $W=0.409$) and males ($\chi^2(2)=18.95$, $p<0.001$, $W=0.592$). Human coaching exceeded control in females ($p=0.005$), while both digital voice ($p=0.011$) and human coaching ($p=0.001$) exceeded control in males. Handgrip peak force was influenced more consistently by visual feedback than coaching modality. Motivation differed substantially across conditions ($\chi^2(2)=49.24$, $p<0.001$, $W=0.665$), with scores highest during human coaching, intermediate during digital voice coaching and lowest during control. Post-task perceived exertion did not differ between conditions.

Conclusions: The effects of verbal coaching on acute physical performance are task-dependent. Standardised digital voice coaching reproduced several performance benefits of live coaching, whereas interpersonal human presence provided an additional motivational benefit.

C12

IL-17A does not potentiate IL-1 β -induced inflammatory responses in human iPSC-derived ventral midbrain astrocytes

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Neuroinflammation is an important component of Parkinson's disease (PD) pathology, where astrocytes are exposed to inflammatory signals derived from resident and infiltrating immune cells. Interleukin-1 β (IL-1 β) can induce astrocyte reactivity, while interleukin-17A (IL-17A), a Th17-associated cytokine reported to be elevated in PD, has previously been shown to synergise with tumour necrosis factor- α (TNF α) in human astrocytes. We therefore investigated whether IL-17A similarly potentiates IL-1 β -induced inflammatory responses in human induced pluripotent stem cell (iPSC)-derived ventral midbrain astrocytes.

NAS2 human iPSC-derived ventral midbrain astrocytes were exposed to cytokines for 24 h. An IL-1 β dose-response study (0.01–3 ng/mL) was first performed to identify submaximal concentrations suitable for detecting cytokine interactions. IL-1 β at 0.01 and 0.1 ng/mL was subsequently tested alone or in combination with IL-17A (10 ng/mL), a concentration previously selected to elicit minimal inflammatory responses when applied alone. Secreted IL-6, CCL5 and CXCL10 were quantified by ELISA, and expression of IL6, CCL5, CXCL10, ICAM1, C3 and GFAP was assessed by quantitative PCR. Astrocyte phenotype and morphology were evaluated by immunocytochemical analysis of GFAP, S100 β , CX43 and EAAT1 and quantitative morphological measurements. Data were analysed using nested ANOVA.

IL-1 β induced strong inflammatory responses, increasing IL-6, CCL5 and CXCL10 secretion and the expression of multiple inflammatory and reactivity-associated genes. Several responses were concentration-dependent. In contrast, IL-17A alone induced limited responses, and its addition to IL-1 β did not consistently increase inflammatory mediator secretion or gene expression. Cytokine treatment for 24 h did not alter cellular morphology or GFAP, S100 β , CX43 and EAAT1 protein immunoreactivity.

These findings demonstrate that IL-1 β induces a strong acute inflammatory response in human iPSC-derived ventral midbrain astrocytes, but this response is not consistently potentiated by IL-17A. Despite changes in inflammatory mediator secretion and gene expression, no overt phenotypic or morphological alterations were detected following 24 h cytokine exposure. Overall, these findings provide no clear evidence of synergistic interaction between IL-1 β and IL-17A under the conditions tested.

C13

Integrating Predicted Efficacy, Specificity and Uncertainty to Prioritise CRISPR Interference Guides for MALAT1 in Lung Adenocarcinoma Cells

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Background and aim

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is implicated in cancer-related gene regulation and is a target for transcriptional modulation in lung adenocarcinoma. CRISPR interference (CRISPRi) guide selection must balance predicted efficacy, local genomic context, sequence feasibility and genome-wide specificity. We developed an uncertainty-aware framework to prioritise MALAT1 CRISPRi guides in A549 cells and inform reproducible cell-physiology experiments.

Methods

Eighty-six guides within ± 500 bp of the MALAT1 transcription-start anchor were assessed using six criteria spanning four domains: target position and chromatin accessibility; local transcript and promoter overlap; sequence feasibility; and predicted CRISPRi specificity. A customised stochastic multicriteria acceptability analysis type-2 framework sampled hierarchical uncertainty in criterion weights over 30,000 Monte Carlo iterations for each of three preference profiles. A profile-free global-weight stress test used 50,000 iterations. Position-model, sequence-quality, off-target-model, leave-one-domain-out, convergence and random-seed analyses assessed robustness.

Results

Candidates 0046, 0047 and 0048 formed a robust core. Their minimum probabilities of ranking in the top five across the three profiles were 0.980, 0.919 and 0.601, respectively; corresponding means were 0.993, 0.969 and 0.807. Under profile-free globally sampled weights, their top-five probabilities were 0.817, 0.741 and 0.519. Candidate 0045 remained competitive but shifted from rank 3 to 29 across off-target models. In one-at-a-time analysis, weight uncertainty produced the largest single mean absolute rank shift (1.49 ranks), compared with off-target-specificity modelling (0.90) and local-context uncertainty (0.64).

Conclusion

Integrating predicted efficacy context and specificity with explicit uncertainty identified candidates that remained competitive under alternative decision assumptions. By distinguishing consistently high-ranked guides from context-sensitive alternatives, the framework can support guide panels for cell-physiology experiments testing how MALAT1 repression affects transcriptional state, proliferation, migration and stress responses. It can also separate guide-selection uncertainty from downstream biological variability, strengthening interpretation and reproducibility.

Ethics

This study used no human participants, identifiable human data, human tissue or animals; research ethics approval was not required.

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Body word count: 300 • Candidate set size: 86 • No biological sample size or inferential p-value applies to this in silico study.

C14

Store-operated Ca^{2+} entry (SOCE) via Orai channels is a key source of Ca^{2+} influx in murine urethral smooth muscle to sustain agonist and nerve evoked contractions

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Urethral smooth muscle (USM) contractions contribute to urethral closure pressure, and are modulated by agonists that liberate Ca^{2+} from intracellular stores via IP3 receptors (IP3Rs), such as phenylephrine (PE), or arginine vasopressin (AVP). Voltage dependent L-type Ca^{2+} channels (LTCC) contribute to USM contractility in numerous species, however a previous study in mice failed to detect any effect of LTCC inhibitors (nifedipine, nicardipine) on male USM contractions induced by PE (Drumm *et al.*, 2018). Conversely, inhibiting store-operated Ca^{2+} entry (SOCE) with Orai channel inhibitors markedly reduced PE responses. However, this study was limited in that only high PE concentrations (10 micromolar) were tested on USM responses and contractions evoked by nerve stimulation were not assessed.

We hypothesised that contributions of LTCC may be apparent at lower agonist concentrations or during nerve stimulation, rationalizing exclusively high agonist doses would cause exaggerated Ca^{2+} release from internal stores and activate SOCE, masking possible contributions of LTCC to lower magnitude responses, thus importance of LTCC in mouse USM contractility under a range of experimental conditions may be underestimated. To test this, we carefully examined SOCE and LTCC contribution to murine USM contractile behaviours.

qPCR revealed male and female USM expressed transcripts for LTCC (*Cacna1a,c,d,f*) and *Orai1-3* paralogs. *Orai1-3* expression was 4-5x greater in both sexes compared to LTCC (n=3). In male USM organ bath experiments, a range of PE concentrations (0.1-30 micromolar) or electrical field stimulation (EFS) at 1, 2, 5, 10 Hz (30 sec), led to dose-dependent and frequency dependent increases in contractile area and amplitude respectively. Nifedipine failed to affect PE or EFS evoked contractions at any agonist concentration (n=6) or EFS frequency (n=6). Sustained female USM contractions in response to 10 nM AVP were unaffected by nifedipine (n=5). FPL64176 (LTCC agonist, 1 micromolar) slightly increased EFS response amplitude by ~10% at frequencies >5Hz, and this was reversed by nifedipine (n=6, 1 micromolar). FPL64176 slightly increased (~10%) PE evoked contractile area of male USM at agonist concentrations of 0.3-30 micromolar (n=6), but did not affect AVP responses in female USM (n=5). All responses to PE, EFS and AVP were abolished by the IP3R antagonist 2-APB, highlighting dependence on sarcoplasmic reticulum (SR) Ca^{2+} release. Inhibition of SOCE with the Orai antagonist GSK 7975A (10 micromolar) significantly reduced PE induced contractile area of male USM (>50%, $P < 0.001$) across agonist concentrations 0.1-30 micromolar (n=3). Amplitudes of EFS evoked contractions in male USM were significantly reduced (~50%) by GSK 7975A (n=13, $P = 0.002 - < 0.001$), and Synta66 (Orai antagonist, 10 micromolar, $P = 0.008 - < 0.001$, n=6). When EFS responses were reduced by either Orai antagonist, further addition of nifedipine caused a 5-20% decrease in amplitude (n=13, $P < 0.001$ (GSK), n=6, $P = 0.001$, Synta66). Both Orai inhibitors significantly reduced sustained contractions evoked by continuous application of 1 micromolar PE in males ($P < 0.01$, n=5) or 10 nM AVP in females ($P < 0.0001$, n=6), and subsequent application of nifedipine had no effect.

We conclude that in mouse USM, Ca^{2+} influx via SOCE through Orai channels, and not via LTCC, is a key Ca^{2+} source required to sustain agonist and nerve evoked contractions.

C15

Evaluation of the effects of a positive allosteric modulator of ANO1 channels (PAM_16A) on mouse gastric and colonic rhythmicity

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Ca²⁺-activated-Cl⁻ channels encoded by ANO1 are exclusively expressed in specialized interstitial cells of Cajal (ICC) in the gastrointestinal (GI) tract muscularis, and serves as the ubiquitous excitatory conductance that generates electrical pacemaker activity and excitatory post-junctional neuronal responses in these cells (Sanders *et al.*, 2026). Activation of ANO1 in ICC leads to depolarization, which is transduced to electrically coupled smooth muscle cells to coordinate regular contractions that underlie GI motility. The importance of ANO1 to normal gut function is demonstrated by the perturbation of normal electrical and contractile behaviours of GI tissues by genetic ablation of ANO1 or selective pharmacological inhibitors of ANO1 (such as Ani9). However, in many GI tract pathophysiological disorders, increasing regular contractions would be desirable (such as delayed gastric emptying or slow transit constipation), however, to date the effects of increasing ANO1 activity on GI tissues to accomplish this has not been characterised. Recently, a small molecule that acts as a positive allosteric modulator of ANO1, PAM_16A, was identified and characterized to enhance ANO1-dependent changes in vascular smooth muscle tone (Al-Hosni *et al.*, 2025). In our study, we sought to evaluate the functional effects of PAM_16A on GI contractility and electrical activity.

We performed isometric tension recordings of circularly orientated strips of mouse gastric antrum and proximal colon (C57/wild-type, 10-16 weeks old), both of which generated spontaneous phasic contractions after an initial application of 5 mN of stretch. In gastric antrum, application of PAM_16A (300 nM - 1 mM) lead to an enhancement in the amplitude of spontaneous contractile amplitude by 42 % (n=5). Similarly, in strips of proximal colon, 300 nM PAM_16A enhanced contractile amplitude by 45% and this was further potentiated to an 80% increase when the concentration of PAM_16A was increased to 1 mM (n=3). In both gastric antrum and proximal colon, application of the ANO1 inhibitor Ani9 (1 mM) significantly reduced contractile activity in the continued presence of PAM_16A (abolishing it in the proximal colon (n=3) and reduced gastric antrum contraction amplitude to 4 % of control (n=x)). In antrum and colon, application of the muscarinic agonist carbachol (CCh, 300 nM - 1 mM) evoked a large amplitude, sustained contraction with superimposed phasic contractions over the length of agonist exposure. In gastric antrum and proximal colon, PAM_16A (1 mM) enhanced the amplitude of the CCh-evoked superimposed phasic contractions by 27 % and 30 % respectively, and these effects were reversed by Ani9, with neither drug affecting the sustained component of CCh responses. In intracellular recordings, spontaneous electrical slow waves were recorded from mouse gastric antrum. Application of PAM_16A (300 nM) accelerated the upstroke component, increasing the initial amplitude of slow waves and caused a slight depolarization of resting membrane potential.

Our preliminary data suggests that the small molecule PAM_16A enhances electrical and contractile activity of mouse gastric antrum and proximal colon, resulting in increased contractile amplitude.

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PAM_16A may be a potentially effective pharmacological tool to probe the effects of ANO1 channel modulation in GI tissues.

C16

Potentiated contractility of mouse proximal colon by the Orai1 enhancer IA65 is caused by increasing Ca²⁺ transient firing in interstitial cells of Cajal

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Background

Colonic motility is regulated by coordinated interactions between electrically coupled smooth muscle cells (SMC) and non-contractile ICC - interstitial cells of Cajal (Sanders *et al.*, 2014). In the gut, ICC exclusively express the Ca²⁺-activated-Cl⁻ channel, ANO1, activated by intracellular Ca²⁺ transients from endoplasmic reticulum (ER) stores (Zhu *et al.*, 2015). ANO1 activation leads to depolarisation in ICC, and increased excitability of electrically coupled SMC, increasing opening of voltage-dependent Ca²⁺ channels and resulting excitation-contraction coupling (Sanders *et al.*, 2024). Ca²⁺ transient firing in ICC must be continually sustained by regular ER Ca²⁺ refilling to prevent store depletion. This is accomplished by store-operated Ca²⁺ entry (SOCE), mediated by interactions between ER-bound STIM proteins and plasmalemmal Orai channels to allow Ca²⁺ influx for ER refilling (Zheng *et al.*, 2018). Much of the evidence for the role of SOCE in ICC derives from pharmacological Orai channel inhibitors such as GSK 7975A, however effects of potentiating Orai channel activity on colonic contractility or behaviours of ICC are unknown.

Results

This study investigated the newly characterised Orai1 modulator IA65 (Azimi *et al.*, 2020) on colonic smooth muscle contractile behaviour in isolated circular muscle strips from mouse proximal colon. In isometric tension recordings, spontaneous contractions developed following equilibration at a frequency of $3.3 \pm 0.8 \text{ min}^{-1}$, with an average amplitude of $1.1 \pm 0.9 \text{ mN}$ and duration of $5.0 \pm 2.5 \text{ sec}$ (n=5, N=9). Following incubation with the Orai1 enhancer IA65 (1 - 3 micromolar), the average amplitude of contractions increased from $1.1 \pm 0.9 \text{ mN}$ to $1.9 \pm 1.2 \text{ mN}$, while frequency decreased by 30.7 % (3 micromolar; n=5, N=9). Potentiated contractile amplitude in the presence of IA65 was reversed by the specific ANO1 antagonist Ani9 (1 μM , n=5) and the Orai channel inhibitor GSK 7975A (10 micromolar; n=4). Application of the muscarinic agonist carbachol (CCh; 1 micromolar) for 5 mins evoked repeatable responses consisting of an initial transient contraction, following by oscillatory contractions superimposed on a decaying baseline (n=5). While incubation with IA65 had no effect on the initial transient component of CCh responses, the second component was dramatically altered, with the baseline more sustained and oscillatory contractions converted to a tonic response, increasing the sustained contraction from 16.75 to 35.5 % (n=5). Using a mouse expressing a genetically encoded Ca²⁺ reporter exclusively in ICC (Kit-iCre-GCaMP6f), we performed whole tissue recordings of Ca²⁺ transient firing in two populations of ICC in proximal colon, intramuscular ICC (ICC-IM) and ICC at the myenteric plexus (ICC-MY). Both ICC were spontaneous active, generating Ca²⁺ transients *in situ*. 3 micromolar IA65 significantly increased Ca²⁺ transient frequency of ICC-IM (P=0.005, n=6, cells=19) and ICC-MY (P=0.005, n=5, cells=10) by 45%.

Conclusion

IA65, a pharmacological enhancer of Orai1 channels, increased Ca^{2+} transient firing in ICC-IM and ICC-MY in mouse proximal colon, leading to increased ANO1 activation in these cells. Enhanced activation of ANO1 increases the excitability of the colonic syncytium, increasing open probability of voltage-dependent Ca^{2+} channels in SMC, resulting in increased contractile amplitude.

C17

Reliable Detection of Bacteria as a Foundation for Studying Host–Microbe Interactions in Physiology

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The presence of bacteria within tumour tissue is increasingly recognised as a potential contributor to cancer biology. Intratumoural microorganisms may interact with epithelial and immune cells, influence inflammatory signalling and local metabolism, and contribute to changes in the tumour microenvironment. Understanding these host–microbe interactions first requires confidence that microbial signals detected by sequencing represent bacteria genuinely present in the tissue.

This is particularly challenging in tumour sequencing datasets because microbial reads usually represent only a very small fraction of the total sequencing data, while the vast majority originate from the human host. Residual human sequences can be incorrectly classified as microbial, creating false signals and potentially leading to incorrect biological interpretation.

To address this problem, we developed HumanFilter, an open-source computational workflow designed to improve removal of human-derived sequencing reads before microbial analysis. HumanFilter combines complementary filtering approaches and sequentially screens reads against multiple human reference resources, reducing the likelihood that residual host sequences are incorrectly assigned to microbial species.

The workflow was evaluated using simulated datasets in which the origin of human and microbial reads was known, allowing us to determine whether stringent host removal could be achieved without losing genuine bacterial sequences. HumanFilter substantially reduced residual human reads while preserving the microbial signal.

We subsequently applied the workflow to whole-genome sequencing data from ten mucinous rectal adenocarcinoma cases with matched normal tissue, focusing on *Fusobacterium*, a bacterial genus associated with colorectal cancer. More rigorous host filtering reduced apparent microbial background in normal tissue while retaining tumour-associated *Fusobacterium* signals.

Importantly, sequencing findings were assessed alongside immunofluorescence analysis of tumour tissue, providing independent evidence for the presence and localisation of *Fusobacterium*. Agreement between sequencing-based detection and tissue-based imaging increased confidence that the bacterial signal reflected microorganisms genuinely present within the tumour rather than artefacts generated by residual human reads.

This distinction is important for physiological studies of cancer. Before investigating whether tumour-associated bacteria influence immune activation, epithelial signalling, inflammatory pathways, cellular metabolism or other features of the local tissue environment, their presence must first be established

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reliably. HumanFilt provides a quality-control framework that strengthens this first step and supports more confident investigation of host–microbe interactions in cancer physiology.

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Evaluating the electrophysiological signatures of infantile spasms in a model of neonatal hypoxia

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Introduction: Infantile spasms (IS), now classified as infantile epileptic spasm syndrome (IESS), is a rare, severe epileptic encephalopathy of infancy with substantial neurodevelopmental morbidity. Its interictal EEG hallmark, hypsarrhythmia, is a chaotic, high-amplitude pattern of irregular slow waves in the delta range (<3 Hz), and hypoxic-ischemic encephalopathy (HIE) is among the most common identifiable causes of IESS, accounting for up to 12% of cases. We propose that HIE-induced stress elevates corticotrophin-releasing factor (CRF) signalling, disrupting GABAergic inhibitory networks and triggering the age-specific onset of IESS; suppression of this pathway likely underlies the efficacy of first-line ACTH therapy. No existing preclinical model combines a clinically realistic HIE aetiology with CRF sensitivity. We therefore developed a novel *ex vivo* model combining neonatal hypoxic injury, pharmacologically induced delta oscillations as a hypsarrhythmia proxy, and CRF challenge, to test whether hypoxia-exposed networks develop IESS-like excitability and stress-sensitivity.

Aims/objectives: (1) Establish a reproducible *ex vivo* model of delta-frequency oscillations in developing mouse cortex, (2) determine whether neonatal hypoxic-ischemic injury alters cortical network activity and parvalbumin-positive (PV+) interneuron density, and (3) test whether hypoxia-exposed networks show increased CRF sensitivity across the postnatal window relevant to IESS (P10–P14).

Methods: All procedures accorded with EU Directive 2010/63/EU and were approved by the Trinity College Dublin Animal Welfare Body, licensed by the Health Products Regulatory Authority (AE19136/I775, AE19136/P140). Neonatal hypoxia was induced in C57BL/6J pups (P7) by exposure to 5% O₂/95% N₂ for 15 min at 34°C; littermate controls received a room-air sham exposure. Motor function was assessed at 24 h and 72 h post-insult. Pups were killed by decapitation for preparation of 400 µm somatosensory cortex slices. Local field potentials were recorded from layer IV/V of the barrel field; delta oscillations were induced with carbachol (2 µM) and SCH23390 (10 µM), followed by CRF (20 nM), and quantified by fast Fourier transform (area under the curve, AUC; peak amplitude; 0.5–4 Hz). Separate cohorts underwent PV immunohistochemistry (P10, P12, P14) and cFos/Arc mRNA quantification (P14). Data are mean ± SEM; comparisons used Mann–Whitney U tests, mixed-effects models, or two-way ANOVA with Šídák's post hoc test; *p*<0.05 was significant.

Results: By 72 h, hypoxia-exposed pups (*n*=6–16 per test) showed reduced ambulation (*p*=0.0286), increased hindlimb foot angle (*p*=0.0007), impaired negative geotaxis (*p*=0.0129) and reduced grip strength (*p*=0.0122) versus controls. Hypoxia slices (*N*=4–5/group) showed increased delta-band AUC and amplitude at P10–P14, at baseline and following CRF (*p*<0.05), with CRF unmasking large,

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hypsarhythmia-like discharges in hypoxia-exposed but not control tissue. PV+ interneuron counts in S1BF (N=3/group/age) were reduced at P12 ($p<0.05$) and P14 ($p<0.0001$), with layer-specific loss at P14 in layers II/III, IV and V ($p<0.01$ – $p<0.001$). cFos and Arc mRNA in P14 cortex did not differ between groups ($n=5$ /group).

Conclusions: Neonatal hypoxia produces persistent behavioural deficits, layer-specific PV+ interneuron loss, and enduring cortical hyperexcitability with heightened CRF sensitivity across a developmental window relevant to IESS. This is the first *ex vivo* model linking a clinically realistic HIE aetiology to CRF-driven, IESS-like network dysfunction, offering a platform for testing CRF-targeted and interneuron-directed interventions.

C19

Ayahuasca-derived harmala alkaloids, harmine and harmaline, both inhibit depolarisation-induced calcium entry in S-type neuroblastoma cells.

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Anecdotal evidence suggests that alkaloids contained within ayahuasca brew, a traditional South American plant-based tea made from the *Banisteriopsis caapi* vine and the *Psychotria viridis* shrub, when used in combination with reconsolidation therapy (RT), may be a novel treatment for psychiatric disorders such as post-traumatic stress disorder (PTSD). Although the mechanism of action underlying this effect remains unclear, we hypothesised that it may be due to effects on neuronal calcium homeostasis. We therefore sought to determine if the ayahuasca-derived harmala alkaloids, harmine and harmaline, modulated depolarisation-induced calcium entry in differentiated neuroblastoma-derived SH-SY5Y cells.

SH-SY5Y cells, initially plated at a density of 1×10^6 cells on glass coverslips for 24 hours, were treated with retinoic acid ($10 \mu\text{M}$) to induce differentiation into a neuronal phenotype. Cells were used for experimentation after the cells had been incubated for further 5-10 days at 37°C , in the presence or absence of either harmine ($1 \mu\text{M}$) or harmaline ($1 \mu\text{M}$). Prior to experimentation, cells were loaded with the non-ratiometric calcium-sensitive dye, Calbryte 520 AM ($4 \mu\text{M}$), for one hour. Coverslips containing the cells were then placed under a microscope and superfused (2 ml/min) with normal HEPES-buffered saline solution (HBSS) for 10 minutes before depolarisation was induced by superfusing on a 50 mM K^+ -containing HBSS solution for 5 mins, followed by a 15 minute washout period. Calcium responses (F/F_0) in individual cells were recorded and measured using the calcium imaging software, Winfluor (John Dempster, Strathclyde University). Both the percentage peak depolarisation-induced calcium response (relative to baseline), and area under the curve (AUC; calculated using Graphpad Prism) for 300 seconds after calcium influx was initiated in untreated (control) neurons, were compared to calcium responses generated in the presence of the harmala alkaloids. Statistical analyses were conducted using one way ANOVA with Dunnett's multiple comparisons test with values of $P < 0.05$ considered statistically significant. All data are expressed as mean $\pm$ S.D.

Our results demonstrated that 50 mM K^+ -HBSS increased intracellular calcium above basal levels by $26.6 \pm 15.8\%$, with an average AUC of 6303.8 ± 4230.6 (arbitrary units (a.u.); $n=36$). Harmine (1 mM) significantly reduced both the peak depolarisation-induced calcium response by 31.4% ($18.3 \pm 8.0\%$ above basal calcium; $n = 30$; $P < 0.05$) and the corresponding AUC by 39.0% (3846.6 ± 1567.0 a.u.; $n = 30$; $P < 0.01$) relative to responses in untreated cells. Similarly, harmaline (1 mM) also significantly reduced both the peak depolarisation-induced calcium response and AUC to an even greater extent than harmine, by 54.4% ($12.1 \pm 10.3\%$ above basal calcium; $n = 29$; $P < 0.0001$) and 63.7% (2289.3 ± 2218.9 ; $n = 29$; $P < 0.0001$), respectively, relative to responses in untreated cells.

Our hypothesis that one possible means by which ayahuasca-derived compounds may alter neuronal function by modulating neuronal calcium homeostasis is supported by our finding that chronic treatment with the ayahuasca-derived harmala alkaloids, harmine and harmaline, significantly reduces depolarisation-induced calcium entry into neuron-like SH-SY5Y cells.

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Acute Effects of Suspension Exercise on Middle Cerebral Artery Blood Velocity Kinetics to Hypercapnia in Healthy Young Adults[

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Background/Aim: The kinetics of the middle cerebral artery blood velocity (MCAv) response to hypercapnia provide insight into cerebrovascular responsiveness and function. Characterising these responses following acute exercise may provide insight beyond conventional steady-state measures of cerebrovascular reactivity. Suspension exercise combines bodyweight movements and instability in a functional resistance modality, presenting a unique haemodynamic challenge. However, the effects of suspension exercise on MCAv kinetics during hypercapnia remain unknown. Therefore, this study aimed to determine the acute effects of suspension exercise on MCAv kinetics during a hypercapnic challenge.

Methods: 9 healthy young adults, 5 male (mean age = 23.4 ± 3 years, $\dot{V}O_{2\max}$ 41.2 ± 9.3 ml/kg/min, BMI 22.9 ± 3.5 kg/m²) were recruited to complete two time-matched visits: a 30-min suspension training session consisting of five different exercises performed in a circuit format, and a 30-min control condition (seated rest). Hypercapnia was induced using a Douglas bag filled with a 6% CO₂ gas mixture (21% O₂, balance N₂) for 4 min at baseline, 10 min, and 60 min post-intervention. Transcranial Doppler ultrasound measured MCAv bilaterally. MCAv Kinetic responses to hypercapnia were analysed using a monoexponential model with a time delay to determine baseline MCAv, the time constant (τ), time delay (TD), mean response time (MRT), and both the absolute and relative response amplitudes. These results were analysed using a two-way analysis of variance.

Results: Baseline MCAv and the time delay (TD) of the MCAv response were unchanged across time and condition (all $P > 0.069$). Although τ differed between conditions ($P = 0.013$), no time or interaction effects were observed ($P \geq 0.411$). The amplitude of the absolute and the relative MCAv responses both had a significant main time $\times$ condition interaction effect (both $P < 0.001$). Absolute and relative MCAv response amplitudes increased 10 minutes following suspension exercise ($P \leq 0.007$), were significantly greater than the control condition (absolute: 31.4 ± 5.9 vs 15.2 ± 5.1 ; relative: 48.1 ± 8.5 vs 24.0 ± 7.6 ; both $P < 0.001$), and returned to baseline by 60 minutes ($P \leq 0.001$).

Conclusion: Suspension exercise transiently enhances the magnitude of the MCAv response to hypercapnia without altering its temporal dynamics, indicating a short-lived improvement in cerebrovascular reactivity. The time-based parameters of the MCAv response (τ , TD, MRT) were maintained, indicating that suspension training affected only the size of the response, not the speed. Given the small sample size of this study, these preliminary findings should be confirmed in future with larger, adequately powered studies.

C21

The effect of 27-hydroxycholesterol on hiPSC-derived APOE3/3 and APOE4/4 astrocytes

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Alzheimer's disease (AD) is a debilitating neurodegenerative disease that causes progressive neuronal death, reductions in brain volume and memory impairment. In the first description of the AD brain, Alois Alzheimer noted the aberrant accumulation of lipid granules in the glia (Foley, 2010). Since then, various aspects of lipid metabolism – such as cholesterol dyshomeostasis – have been linked to AD development and progression (Delac & Maioli, 2025). Cholesterol itself cannot cross the blood brain barrier, however its metabolism into oxysterols allows the bi-directional flux of these lipids between the brain and peripheries. 27-hydroxycholesterol (27-OHC) is the most abundant oxysterol in the circulation and evidence suggests it can exacerbate AD-related cellular pathology and impact memory and cognition. The APOE gene, which encodes for a multifunctional protein involved in lipid transport and homeostasis, is also highly implicated in AD development (Raulin et al., 2022). APOE is highly expressed in the brain's astrocyte population, and the isoform present significantly influences AD risk, pathology and outcomes.

This research seeks to understand if 27-OHC exposure influences astrocyte health and metabolism in an APOE isoform-dependent manner, potentially providing insight into isoform-related differences in AD risk.

APOE3/3 and APOE4/4 hiPSC-derived astrocytes were used throughout the study; with these genotypes representing the most common, “neutral” risk polymorphism and the rarer, high-risk polymorphism respectively (Raulin et al., 2022). Mass spectrometry analysis was performed to assess the levels of total cholesterol and various oxysterols, including 27-OHC, at baseline levels. Cell metabolic capacity in response to 27-OHC (0-10µM) exposure was recorded in both genotypes using the CellTiter-Blue® assay. Additionally, immunofluorescent staining was used to investigate changes in mitochondrial membrane potential and nuclei-associated parameters.

Mass spectrometry analysis successfully detected 27-OHC in both genotypes, with preliminary results suggesting that APOE4/4 cells contain over two-fold the levels of 27-OHC than their APOE3/3 counterparts (n=1). Our results also indicate that 27-OHC has a proliferative or protective effect on cells of both genotypes (n= 3-4). Furthermore, preliminary evidence suggests that 27-OHC does not affect mitochondrial membrane potential or influence nuclear area or intensity (n=1). However, an upward trend in nuclei number was observed in APOE4/4, but not APOE3/3 cells, upon exposure to increasing concentrations of 27-OHC. This provides further support to the 27-OHC's potential proliferative/protective role.

Previously, 27-OHC has been evidenced to increase proliferation in other cell types – particularly in cancer cells (Hou et al., 2025). However, to this author’s knowledge, this is the first indication it may exert a proliferative effect on astrocyte populations. Further work must be done to elucidate the mechanisms underlying this effect. Additionally, building on the research of this study will offer insight into potential differences in lipid metabolism and cellular responses between APOE3/3 and APOE4/4 astrocytes. This, in turn, may be able to deepen the field’s understanding of the relationship between lipid metabolism and AD risk.

NOTE: at time of writing, limited replications have been completed for some experiment (clearly stated as preliminary findings). By the time of presentation, it is expected each experiment will have a minimum of n=3.

NOTE: at time of writing, limited replications have been completed for some aspects of the study. By the time of presentation, it is expected each experiment will have a minimum of n=3.

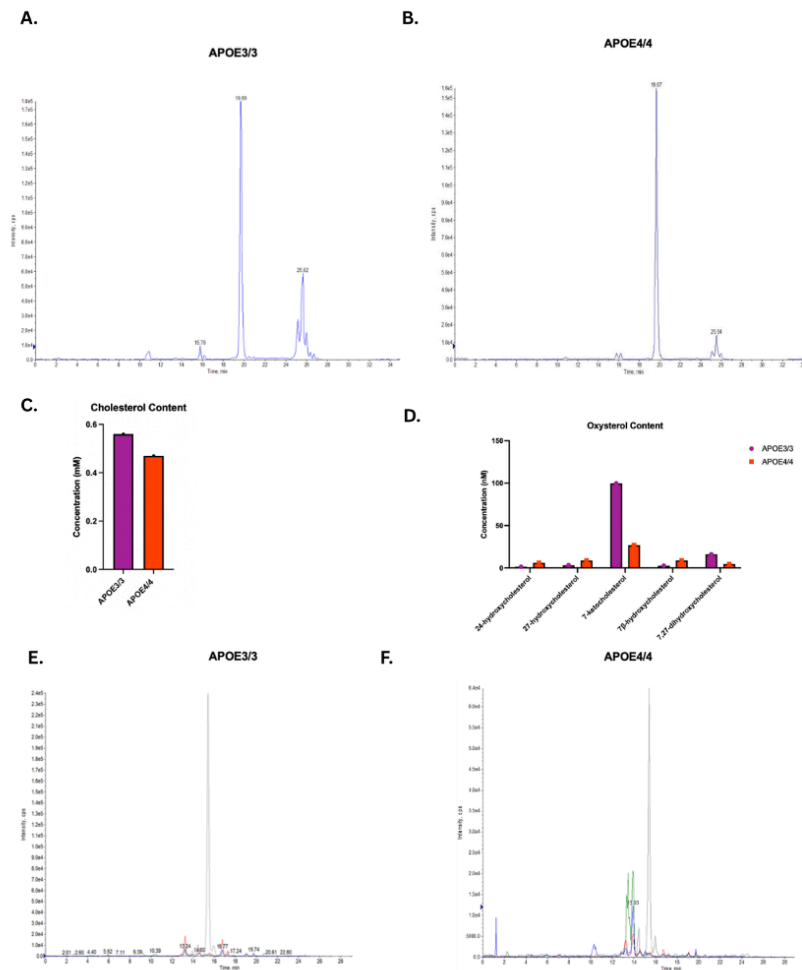


Fig 1. Cholesterol and oxysterol content of APOE3/3 and APOE4/4 hiPSC-derived astrocytes. Untreated hiPSC-derived astrocytes of both genotypes underwent a lipid extraction protocol, and the resulting extracts were prepared and subjected to mass spectrometry analysis. **(A-C)** Cholesterol content in each sample was recorded and quantified. **(D-F)** Levels of several disease-relevant oxysterols in each sample were also evaluated during analysis. N = 1 technical replicates from one independent experiment per genotype – therefore no statistical analysis has yet been performed.

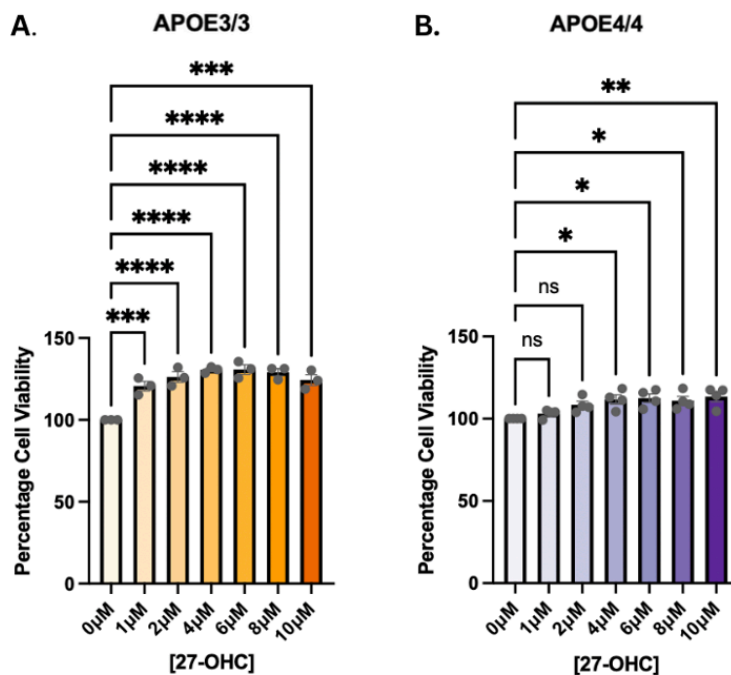


Fig 2. 27-OHC treatment increases cell metabolic capacity. hPSC-derived astrocytes were treated with varying concentrations of 27-OHC (0-10μM) for 24h. Cell viability, assessed using the cell titer blue assay, was evaluated in (A) APOE3/3 and (B) APOE4/4 cells. Data are expressed as mean ± SEM. N = 9-12 technical replicates, from 3-4 independent experiments per genotype. Each point represents the mean value from 3 technical replicates. In the APOE3/3 assay, technical replicates were no greater than 10% on either side of the group mean. In the APOE4/4 assay, technical replicates were no greater than 15% on either side of the group mean. Statistical analysis was performed in GraphPad Prism using a one-way ANOVA followed by the Tukey post-hoc test. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

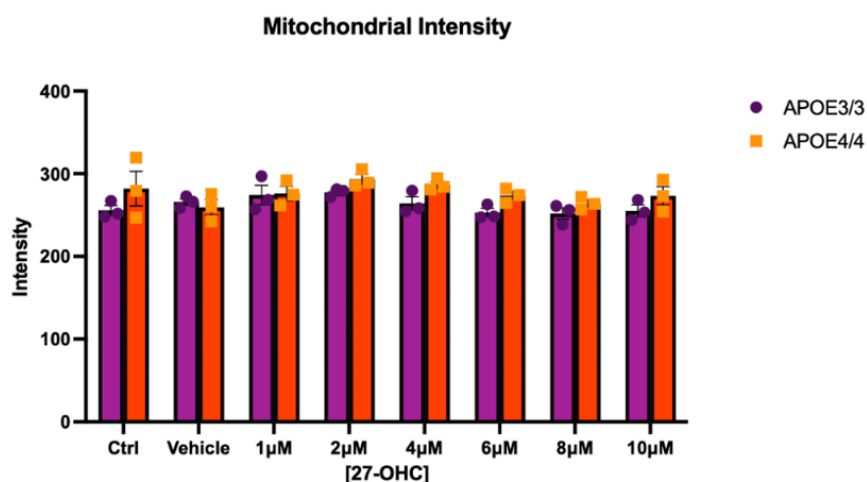


Fig 3. 27-OHC does not affect mitochondrial membrane potential. hPSC-derived astrocytes were treated with varying concentrations of 27-OHC (0-10μM) for 24h before staining with TMRM to assess mitochondrial membrane potential. The intensity of TMRM staining in APOE3/3 and APOE4/4 astrocytes was quantified. N = 3 technical replicates from one independent experiment per genotype therefore statistical analysis has not yet taken place. Each point on the bar graph represents one technical replicate. Image analysis was performed using a combination of ImageJ and Python.

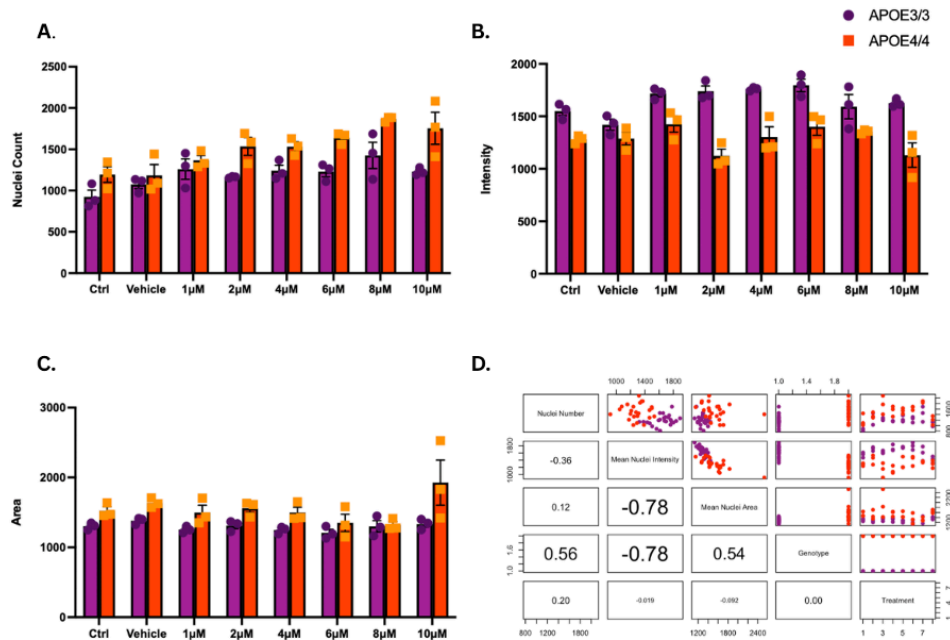


Figure 4. Investigating nuclear parameters in APOE3/3 and APOE4/4 hiPSC astrocytes in the presence of 27-OHC. hiPSC-derived astrocytes were treated with a range of 27-OHC concentrations (0-10μM) for 24h. Cells were treated with Hoechst dye and live-imaged immediately afterward. Images were computationally analysed to assess (A) nuclei number, (B) area and (C) intensity in both genotypes. (D) Pairwise partial correlations were performed between the nuclei number, nuclei area, nuclei intensity, treatment group and genotype were performed. N = 3 technical replicates from one independent experiment per genotype - therefore statistical analysis did not take place. Image analysis was performed using a combination of ImageJ and Python. Pairwise partial comparisons were calculated and visualised in R.

C22

Multimodal preparation in cardiovascular physiology: a mixed-methods evaluation across three semesters

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Introduction. Learning valvular disorders and murmurs requires students to integrate cardiac-cycle events, intracardiac pressure gradients, blood flow, valve mechanics, and auscultatory findings. Multimodal preparatory resources may facilitate this integration before team-based learning (TBL), although excessive preparatory volume may increase student workload.

Aim. To evaluate students' perceptions of multimodal preparatory resources, assessment alignment and faculty-facilitated learning during a clinically integrated cardiovascular physiology TBL session.

Methods. Post-session evaluation data were analysed retrospectively across Spring, Summer and Fall 2023 semesters. The preparatory package comprised detailed handouts, instructional videos, summary slides, interactive workbooks, clinical cases, practice questions and flashcards. Quantitative items assessed the quality and quantity of the materials, resource use, relevance and difficulty of readiness assurance test (RAT) questions and application exercises, identification of knowledge gaps, and faculty facilitation. Data were summarised using frequencies, percentages, medians and interquartile ranges. Independent cohorts were compared using Kruskal-Wallis tests. Free-text responses were analysed thematically. The study received institutional ethical approval.

Results. Of 659 eligible students, 597 completed the primary evaluation item (90.6%; Spring n=301, Summer n=157 and Fall n=139). Overall, 451/597 students (75.5%) rated the preparatory materials as high or very high quality (median 2, interquartile range 1–2; 1=very high and 5=very low), with no statistically significant difference among semesters ($H(2)=2.60$, $p=0.273$). Instructor-provided resources were used by 558/592 students (94.3%). RAT questions were rated relevant or highly relevant by 530/591 students (89.7%), and application exercises by 516/591 students (87.3%). Ratings did not differ significantly among cohorts for RAT-question relevance ($p=0.077$) or application-exercise relevance ($p=0.663$). Although 376/591 students (63.6%) considered the quantity of preparation appropriate, 206/591 (34.9%) considered it excessive. Most students agreed or strongly agreed that the session identified knowledge gaps (440/591, 74.5%) and that faculty-led discussion filled knowledge gaps and reinforced learning (432/591, 73.1% for each item). Faculty facilitation was perceived as engaging by 403/591 students (68.2%). Qualitative themes included clarity and organisation, flexibility through multiple learning modalities, clinical integration and readiness for assessment. The principal concern was the density of the materials and the time required to complete them.

Conclusion. Purpose-built multimodal resources were consistently perceived as high quality and closely aligned with assessment and clinical application in cardiovascular physiology. Their educational value may depend on balancing comprehensiveness with a manageable preparatory workload. Further research should examine relationships between resource use, objective performance, and longer-term retention.

C23

Neonatal Hypoxia Reprograms Neuroimmune Responses to Later-Life Inflammation

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Hypoxic-ischaemic encephalopathy is a leading cause of neonatal neurological injury, with neuroinflammation contributing to both disease progression and long-term impairment (Tetorou et al., 2021). Neonatal hypoxia induces peripheral immune infiltration and alters glial activation within the developing brain (Li et al., 2017). However, it remains unclear whether early-life hypoxic injury reprograms the neuroimmune response to subsequent inflammatory challenges later in life. This study characterised acute peripheral immune infiltration following neonatal hypoxia and assessed whether prior hypoxic injury alters inflammatory signalling, myeloid recruitment, and microglial phenotype after a subsequent peripheral immune challenge.

C57BL/6J pups were subjected to global hypoxia (5% O₂/95% N₂) or normoxia for 15 mins on postnatal day 7. Brains were collected at 3, 6, 24, or 72 hours post-hypoxia, and CD45⁺ immune populations were quantified by flow cytometry (n = 4–6 per group per time point). Additional cohorts also received lipopolysaccharide (0.3 mg/kg, i.p.) or saline at P21. Cortex, hippocampus, and dura mater were collected 3 hours post-injection for flow cytometry, ELISA, and qPCR (n = 3–6 per group), and 24 hours post-injection for immunohistochemistry (n = 3–4 per group). Microglial morphology was quantified by Iba1 immunofluorescence and analysed using FIJI with unsupervised clustering. All procedures were approved by the Trinity College Dublin Animal Research Ethics Committee and performed under authorisation from the Health Products Regulatory Authority in accordance with European Union Directive 2010/63/EU and S.I. No. 543 of 2012; animals were culled by transcardial perfusion under terminal pentobarbital anaesthesia. Data are presented as mean ± S.E.M. and were compared by unpaired t-test or two-way ANOVA with Tukey's multiple comparisons test.

Neonatal hypoxia increased brain neutrophils at 6 hours, CD8⁺ T cells at 6 and 24 hours, CD4⁺ T cells at 24 hours, and B cells at 6 and 24 hours (all $P < 0.05$). Following lipopolysaccharide administration at P21, hypoxia-exposed mice exhibited fewer cortical monocytes ($P < 0.01$) and macrophages ($P < 0.05$) compared to challenged controls, while dura mater populations remained unchanged. This attenuated recruitment occurred despite elevated cortical IL-1 β ($P < 0.001$), markedly lower CCL2 ($P < 0.01$), and reduced expression of the endothelial activation marker *Sele* ($P < 0.0001$). Morphometric clustering identified homeostatic, hyper-ramified, and reactive-like microglia. After challenge, hypoxia-exposed mice had fewer homeostatic microglia in the cortex ($P < 0.05$) and a shift toward a hyper-ramified phenotype ($P = 0.068$).

C24

Investigating the interplay between oxygen dysregulation and subsequent immune stress on autonomic function in a neonatal rat model.

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Background: Preterm infants born before 32 weeks frequently experience chronic intermittent hypoxia (CIHH) due to cardiorespiratory instability and are at heightened risk of nosocomial gram-positive infection. Both stressors independently compromise blood-brain barrier (BBB) integrity and activate neuroinflammation, yet their combined hypothalamic effects in early life remain poorly understood.

The paraventricular nucleus (PVN) is a critical integrator of the stress response, coordinating HPA axis activation via corticotrophin-releasing hormone (CRH), sympathetic output via tyrosine hydroxylase (TH)-positive, and release of oxytocin (OXT) and arginine vasopressin (AVP).

AIM: Using a neonatal rat model of chronic oxygen dysregulation and subsequent acute gram-positive bacterial challenge (LTA+PGN), we sought to characterise the model examining the potential autonomic changes associated with the stressors.

Methods: Study was conducted following HRPAA approval and in line with European guidelines. Dams and litters were either raised at room air or placed in an environmental chamber where FiO₂ was manipulated to generate intermittent hypoxia (8% FiO₂)/hyperoxia (30% FiO₂)/normoxia (21% FiO₂) (CIHH) between postnatal day (PND) 3 and 12. On PND13, pups received intraperitoneal saline or LTA+PGN. Animals were assigned to different experimental assessments a) plasma hormone analysis (multiplex assay), b) plasma cytokine analysis (multiplex assay), c) BBB permeability (Fic-dextran extravasation) e) ultrasonic vocalisation monitoring in vivo (Ultravox and DeepSqueak). Brains from group (e) were harvested for neural characterisation by immunofluorescence for OXT, CRH, AVP, TH and c-Fos. Data was analysed using 3-way ANOVA.

Results: LTA+PGN challenge increased plasma inflammatory cytokines, with IL-1 β , IL-6, and IL-10 showing significant synergistic effects on concentrations when pre-exposed to CIHH. LTA+PGN elevated circulating progesterone and cortisol concentrations. LTA+PGN increased the extra/intravascular fluorescence ratio in the magnocellular and intermediocellular regions of the PVN (Gas $\times$ LTA+PGN, $p=0.02$ and $p=0.01$ respectively). Preliminary histological data ($n=3$) illustrate that OXT⁺ and CRH⁺ cell numbers were unaffected by treatment or sex. In the magnocellular region, c-Fos⁺ cell counts and percentage of c-Fos⁺ OXT⁺ neurons were increased with CIHH exposure (gas;). In the intermediocellular region, c-Fos⁺ cell counts are increased with CIHH () and LTA+PGN (), while the percentage of c-Fos⁺ CRH⁺ neurons were increased in males LTA+PGN (interaction LTA+PGN $\times$ Sex) in sex and LTA+PGN conditions. USV features did not differ between experimental groups.

Conclusions: Increased PVN permeability may facilitate cytokine access to the hypothalamus and contribute to increased neuronal activation observed. Yet, despite evidence of inflammatory, vascular, and neuronal responses, data suggest that these interventions did not alter USV behaviour, suggesting that these stressors may activate hypothalamic pathways without producing measurable autonomic changes at this developmental stage.

C25

Modulation of contractions and cytosolic Ca^{2+} by cAMP and its downstream effector proteins, EPAC and PKA

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Penile erection is mediated by relaxation of corpus cavernosum smooth muscle (CCSM), primarily driven by cyclic nucleotide signalling. Current therapies for erectile dysfunction (ED) target the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) axis, with phosphodiesterase-5 inhibitors (PDE5Is) enhancing cGMP signalling to promote relaxation. However, 30-40% of patients, particularly those with diabetes or vascular disease, respond poorly due to impaired NO bioavailability and reduced cGMP formation, limiting efficacy [1].

The parallel cyclic adenosine monophosphate (cAMP) pathway represents an alternative target, traditionally attributed to protein kinase A (PKA)-mediated reductions in intracellular Ca^{2+} . Clinically, this is exploited via intracavernosal prostaglandin E1 (PGE1; alprostadil). PKA also modulates ion channels, including Kv7.5 channels, expressed in mouse CCSM cells [2]. More recently, exchange protein directly activated by cAMP (EPAC) has emerged as a distinct cAMP effector, inducing smooth muscle relaxation via Rap1/2 signalling, though its role in CCSM remains poorly defined.

Using selective activators of PKA (6-MB) and EPAC (007-AM), we investigated their contributions in mouse CCSM. EPAC-1 expression was confirmed at transcript (RT-PCR, qPCR) and protein levels (immunoreactivity in isolated CCSM cells), with antibody validation in mouse kidney cortex, which highly expresses EPAC-1 [3].

In isometric tension studies, CCSM pre-contracted with phenylephrine (PE; 3×10^{-7} M) exhibited sustained phasic activity. EPAC activation (007-AM, 1×10^{-5} M) produced modest but significant reductions in contraction frequency ($6.87 \pm 1.47 \text{ min}^{-1}$ to $5.17 \pm 1.07 \text{ min}^{-1}$; $P=0.0156$) and amplitude ($0.99 \pm 0.16 \text{ mN}$ to $0.86 \pm 0.16 \text{ mN}$; $P=0.0276$). Similarly, PKA activation (6-MB, 1×10^{-4} M) reduced contraction frequency ($6.70 \pm 0.59 \text{ min}^{-1}$ to $4.57 \pm 0.47 \text{ min}^{-1}$; $P=0.0001$). However, co-activation abolished phasic contraction frequency ($5.83 \pm 0.94 \text{ min}^{-1}$ to $0.00 \pm 0.0 \text{ min}^{-1}$; $P=0.0016$) and amplitude ($0.87 \pm 0.14 \text{ mN}$ to $0.00 \pm 0.0 \text{ mN}$; $P=0.0015$).

At the cellular level, PE induced Ca^{2+} oscillations in isolated CCSM cells. 007-AM significantly reduced oscillation frequency ($20.0 \pm 4.0 \text{ min}^{-1}$ to $0.5 \pm 0.5 \text{ min}^{-1}$; $P=0.0087$) at high PE (1×10^{-5} M), previously identified as membrane potential-independent [2]. In contrast, 6-MB had no effect under these conditions ($25.7 \pm 4.2 \text{ min}^{-1}$ to $25.2 \pm 3.5 \text{ min}^{-1}$; $P=0.8560$) but abolished oscillations ($9.5 \pm 0.9 \text{ min}^{-1}$ to $0.0 \pm 0.0 \text{ min}^{-1}$; $P=0.0445$) at low PE (1×10^{-7} M), which are membrane potential-sensitive. This effect was prevented ($8.0 \pm 1.0 \text{ min}^{-1}$ to $15.5 \pm 2.1 \text{ min}^{-1}$; $P=0.4896$) by the Kv7 blocker XE-991, implicating Kv7 channels in PKA-mediated responses.

Collectively, these data show that EPAC-1 is expressed in CCSM and inhibits Ca^{2+} signalling, potentially via suppression of sarcoplasmic reticulum Ca^{2+} release, while PKA may promote relaxation through Kv7.5 channel activation and membrane hyperpolarisation. Although co-application of 007-AM and 6-MB abolished contractions, the ability of EPAC activation alone to suppress Ca^{2+} oscillations suggests a dominant role for EPAC rather than simple additive effects. Targeting cAMP effectors, particularly EPAC, alone or alongside PKA, may therefore offer a strategy to restore CCSM relaxation in patients unresponsive to PDE5Is.

C26

Alterations in Microglial Gene Expression Following Neonatal Hypoxic-Ischemic Injury

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Neonatal hypoxic-ischemic encephalopathy (HIE) is a serious condition in which the developing brain does not receive enough oxygen and blood flow during the perinatal period. It is a leading cause of infant mortality and the development of later-life sequelae, including cerebral palsy, learning disabilities, and paediatric epilepsy. Microglia, the resident immune cells in the central nervous system, are key drivers of neuroinflammation in response to injury. However, they also play an essential role in shaping neural networks during development, suggesting that microglial dysfunction may link acute injury to long-term neurological deficits. Here, publicly available single-cell RNA-seq data from a mouse model of maternal immune activation and hypoxia were analysed to evaluate changes in microglial gene expression. Analysis was performed in R using the Seurat package. A range of genes were found to be differentially expressed between normoxic and hypoxic microglia. Notably, *Slc1a3*, the gene encoding the glutamate transporter known as GLAST, was lower in hypoxic microglia ($p = 1.54 \times 10^{-35}$). Moreover, the GABAB receptor subunit encoded by *Gabbr1* was also decreased in hypoxic microglia ($p = 0.002$). Pathway enrichment analysis of upregulated genes in the normoxic cells revealed several neurotransmission-related processes, including neurotransmitter receptors and postsynaptic signal transmission ($p = 2.06 \times 10^{-5}$). These findings suggest that genes involved in neuronal signalling, such as glutamate transporters and GABA receptor subunits, are relatively reduced following hypoxia. Overall, hypoxia may shift microglial gene expression away from homeostatic, synapse-related functions. Ultimately, a better understanding of the microglial response to HIE could inform new therapies.

C27

Contractility of mouse myometrium is altered by pharmacological modulators of store-operated-Ca²⁺ entry (SOCE)

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Background

During pregnancy, myometrium smooth muscle cells (SMC) relax to accommodate growth of a developing foetus and during labour, develop regular contractions allowing movement of the foetus towards the cervix (Malik *et al.*, 2021). Irregular myometrium contractions during pre-term labour, leads to complications (foetal heart abnormalities, foetal hypoxia) that present unacceptable risks to infant and maternal mortality (Wray & Prendergast, 2019). Clinically, during pre-term labour or uterine hyperstimulation, myometrium contractions are reduced by drugs such as nifedipine, which block voltage-dependent L-type Ca²⁺ channels, preventing Ca²⁺ entry and contraction. However, nifedipine has numerous effects throughout the body (decreased blood pressure, heart palpitations, etc.) that pose additional risks to mother and foetus. In SMC, the sarcoplasmic reticulum (SR) releases Ca²⁺ to enhance contraction. In many tissues, SR refilling occurs when STIM proteins on the SR membrane activate Orai channels in the plasma membrane, allowing store-operated-Ca²⁺-entry (SOCE) and SR refilling. While previous studies suggest myometrium SMC express STIM / Orai (Chin-Smith *et al.*, 2014) and are capable of SOCE (Noble *et al.*, 2014), effects of specific modulators of SOCE on myometrium have not been tested.

Results

Using isometric tension recordings of myometrium from non-pregnant wild-type mice (10-16 weeks old), we investigated contributions of SOCE to contractile activity in longitudinal strips isolated from cervical and ovarian regions across different stages of estrous. When myometrium strips (N) equilibrated in an organ bath after application of 10 nM oxytocin and 5 mN of initial stretch, spontaneous contractions developed in cervical and ovarian tissue at all stages of estrous (n=37, N=39), with an average frequency of $2.2 \pm 0.7 \text{ min}^{-1}$ and amplitude of $4.5 \pm 2.5 \text{ mN}$. Inhibition of L-type Ca²⁺ channels with nifedipine (1 micromolar) abolished contractions, while addition of Orai channel inhibitors (GSK 7975A, BTP2, Synta66; 10 micromolar) reduced contraction frequency by ~ 40% (n=37, N=39) with minimum effect on amplitude. Greater sensitivity to Orai channel inhibitors was noted in cervical tissues (~ 49% decrease in frequency in cervical tissue (n=37, N=39) vs ~ 31% reduction of frequency in ovarian tissue (n=37, N=39)). Additionally, non-estrous tissue showed greater sensitivity to Orai inhibition compared to estrous tissue from both regions (for example ~ 48% reduction in frequency in non-estrous tissue (n=38, N=40) vs 32% reduction in frequency in estrous tissues n=36, N=38). When SOCE was evoked in myometrium by reintroduction of extracellular Ca²⁺ following SR depletion with Ca²⁺ free solutions and blocking the SERCA pump with thapsigargin (10 micromolar), a prolonged transient contraction occurred. Under these conditions, the Orai channel enhancer IA65 (1 - 3 micromolar) evoked phasic contractions superimposed on a tonic contraction. Inhibition of Orai channels with GSK 7975A reduced both tonic and phasic components, while nifedipine only abolished phasic contractions, with no effect on tone. Furthermore, IA65 (1 and 3 micromolar) increased spontaneous contractile duration in both cervical and ovarian regions (n=6, N=18).

Conclusions

Collectively, these findings support a regulatory role for SOCE in spontaneous myometrial contractility and that degrees of sensitivity to SOCE modulation depend on uterine region and stage of estrous being targeted.

C28

Evaluating the spatial inflammatory profile of neonatal hypoxia and its contributions to neurological outcomes

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Neonatal hypoxia is a medical condition resulting from the deprivation of oxygen to a newborn infant lasting long enough to cause harm, usually to the brain. It remains a serious condition that causes significant mortality and morbidity, including seizures. Outcomes for babies suffering from neonatal hypoxia and seizures are particularly poor, with a 23% mortality rate (1 million neonatal deaths per year worldwide) and survivors developing long-lasting neurological disorders (e.g., cerebral palsy, epilepsy, learning disabilities). Current diagnostics and treatment have limitations; it is an unmet medical need to better understand the pathophysiology of neonatal hypoxia to find novel diagnostic tools that can support more effective treatments. Magnetic resonance imaging (MRI) is the gold standard for the assessment of brain injury in infants with neonatal hypoxia and has been used to characterise the effect of neonatal hypoxia in mouse models which include an ischemic element. However, to date no work has been completed to characterise these effects in a purely hypoxic model.

We evaluate the spatial and temporal pathophysiology of the brain using a mouse model of neonatal hypoxia. At postnatal day 7 (P7), pups are placed in a hypoxia chamber at 34°C and are subjected to hypoxic (5% oxygen / 95% nitrogen) or normoxic (room air) conditions for 15 minutes. Two weeks post-hypoxia (P21), all mice undergo gelatine training for 2-3 days. One group of hypoxia-exposed mice are fed jellies containing an anti-inflammatory compound (MCC950, 75 mg/kg) twice a day for five days, while all others receive control jellies (n=12 per group). Between P28 and P40, mice perform a battery of behavioural tests, including the light/dark box, open field, and novel object location. 5 weeks after hypoxia, mice receive a 0.4 mmol/kg dose of manganese chloride (MnCl₂) and 24 hours later T1- and T2-weighted scans are obtained using a 9.4T MRI scanner. All statistical comparisons between groups use a Kruskal-Wallis non-parametric test.

We evaluate anatomical changes over time after hypoxia and compare them with control and MCC950-treated littermates. T1-weighted scans do not show a change in volume in the cortex or hippocampus, while T2-weighted images show an increase (p=0.0271) in the volume of cytotoxic oedema particularly in the hippocampus, showing that the inflammatory process may be persistent over time. Vehicle-treated hypoxia mice display significant increases in latency to exit the light chamber (p=0.0063) and open field immobility (p=0.0132) relative to controls, as well as a decrease in time spent with the object in the novel location (p=0.0039). This level of impairment strongly correlates with the volume of oedema observed in the hippocampus. MCC950-treated hypoxia mice display no significant behavioural or anatomical differences relative to either group, but their behavioural phenotype is more similar to that of controls.

In conclusion, neonatal hypoxia has profound and lasting consequences in the mouse brain characterised by hippocampal inflammation, which leads to behavioural impairments and long-term neurodevelopmental deficits. Treating hypoxia-exposed mice with MCC950 two weeks post-hypoxia has

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shown indications of reducing this inflammation and subsequent behavioural impairment, but a larger sample size is still required to provide greater statistical power.

C29

Spatial Electrophysiological Mapping of Tumour and Peritumoural Cortex During Glioma-Related Epilepsy Surgery

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Abstract:

Introduction

Intraoperative electrocorticography (ioECoG) enables direct recording of cortical electrical activity and can assist identification of epileptogenic cortex during epilepsy surgery^{1, 2}. High-frequency oscillations (HFOs), including ripples and fast ripples, have emerged as potential complementary biomarkers to interictal spikes³⁻⁶. However, their spatial distribution within glioma, peritumoural and macroscopically normal cortex, and their relationship with subsequent tissue resection and seizure outcome, remain incompletely understood.

Aims and Objectives

This prospective study aims to characterise the spatial electrophysiological profile of tumour, peritumoural and normal cortex during glioma-related epilepsy (GRE) surgery. Specifically, the distribution of HFOs, interictal spikes and delta, theta, alpha and beta activity will be mapped to anatomically defined tissue regions using intraoperative neuronavigation. Electrophysiological characteristics will subsequently be related to tissue resection and 12-month postoperative seizure outcome to investigate whether specific electrophysiological features, and their resection, are associated with seizure outcome.

Methods

Patients with GRE undergoing glioma resection are prospectively recruited. ioECoG is recorded using an 8-contact subdural strip electrode (DIXI Medical) and Xltek Quantum EEG system at a sampling rate of 2048 Hz. A reproducible three-position electrode-placement protocol was developed to achieve systematic spatial sampling using an electrode routinely employed for functional cortical mapping. The strip is positioned sequentially at three cortical locations, rotating 60° around a central reference position, with two minutes of continuous ioECoG recorded at each position.

Neuronavigation snapshots are acquired at predefined strip contacts at each position, supplemented by intraoperative photographs incorporating identifiable vascular or gyral landmarks. Individual recording contacts are subsequently localised on three-dimensional brain reconstructions and classified as tumour, peritumoural or normal cortex.

Recordings are analysed for interictal spikes, ripples (80–250 Hz), fast ripples (250–500 Hz), and delta, theta, alpha and beta activity. Postoperative imaging delineates the resection cavity, allowing the resection status of spatially mapped tissue to be determined. Electrophysiological characteristics and their resection will subsequently be related to 12-month seizure outcome.

Results

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Seven patients (N=7) with low-grade glioma have been recruited and analysed to date. HFOs were identified in two patients (2/7), while interictal spikes were identified in four (4/7), involving tumour, peritumoural and/or normal cortex. Two ioECoG recordings were performed in awake patients (2/7) and five under steady-state propofol anaesthesia (5/7). Spatial and frequency-band analyses are ongoing, together with prospective assessment of postoperative seizure outcome.

Conclusion

Systematic spatial ioECoG sampling across glioma, peritumoural and normal cortex is feasible using a reproducible three-position strip-electrode protocol. Preliminary findings demonstrate HFOs in 2/7 and interictal spikes in 4/7 patients. Integration of spatial electrophysiological characteristics with resection mapping and 12-month seizure outcome will determine whether specific electrophysiological features, and their resection, have potential clinical utility in GRE surgery.

Ethical approval

Beaumont Hospital Medical Research Ethics Committee approval REF25/06

C30

Characterisation of Skeletal Muscle Pathology in the *Ctns* Knockout Rat.

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Background:

Cystine accumulation occurs in all tissues in cystinosis and leads to progressive multisystem pathology, including skeletal muscle dysfunction. Cystinosis-associated myopathy can cause muscle weakness, dysphagia, and pulmonary impairment, contributing to reduced quality of life, loss of independence, and feeding difficulties. Despite its clinical significance, the mechanisms underlying muscle involvement remain poorly understood. While myopathy has been well characterised clinically and in mouse models, the presence of a comparable phenotype in the *Ctns* knockout (KO) rat remains unclear. This study aimed to determine whether the *Ctns* KO rat develops a skeletal muscle phenotype consistent with cystinosis-associated myopathy.

Methods:

Animals were bred in line with European Regulations and with approval from the HPRA and local Animal Ethics and Experimentation Committee at University College Cork (AEEC). A 2 × 2 factorial study design was used with sex and genotype as fixed factors in six-month-old animals ($n = 4-9$ per group). Animals were killed by Schedule 1 method and *ex vivo* isotonic and isometric contractile measurements were performed on isolated soleus and diaphragm muscle strips. Structural analyses were conducted using haematoxylin and eosin and Sirius red staining, while immunohistochemical fibre typing was used for morphometric analysis. Data were analysed using ANOVA in GraphPad Prism.

Results:

Ctns KO animals showed significant reductions in power under 50% load conditions in both soleus and diaphragm. Isometric performance was also impaired, with reduced maximum specific force measured in response to 150Hz. There was no effect of genotype on rate of fatigue. Structural analyses demonstrated preserved muscle architecture, with no evidence of necrosis, inflammation, fibrosis, or fibre type remodelling at 6-months of age.

Conclusion:

Significant functional deficits in isotonic and isometric contractile performance were identified in the soleus and diaphragm muscles of *Ctns* KO rats despite preserved muscle structure. These findings suggest that functional impairment precedes detectable structural degeneration in cystinosis and support the *Ctns* KO rat as a valuable model for investigating neuromuscular involvement in this disease. The diaphragm findings are particularly important given the respiratory complications reported in nephropathic cystinosis.

C31

Store-operated calcium entry in primary mouse hippocampal neurons is inhibited by microtubule stabilisation

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One of the primary means of loading the main neuronal calcium store, the endoplasmic reticulum (ER), is *via* neuronal store-operated calcium entry (nSOCE). As such, nSOCE likely plays an important role in regulating synaptic plasticity and spine growth, and its chronic dysregulation may also play a role in neurodegenerative conditions such as Alzheimer's disease.

A key feature of nSOCE is that its initiation is dependent upon a rapid and significant degree of intracellular rearrangement between the ER and plasma membrane. However, the mechanisms regulating this rearrangement in neurons remain unclear. Previous studies have examined non-excitable cells have reported a role for microtubules in regulating SOCE activation, but it has not yet been established if a similar SOCE mechanism occurs in neurons. The current study therefore investigated whether microtubule stabilisation or depolymerisation played a role in regulating neuronal SOCE in cultured hippocampal neurons.

Cultured primary hippocampal neurons were prepared from 3-5 day old C57BL/10 mice on glass coverslips, and used for experimentation between 7-12 days in culture. Neurons were. Experiments were conducted by first loading neurons with the calcium-sensitive dye, Calbryte 520 AM (4 μ M) for 1 hour. Neurons were then superfused (2ml/min) with Ca²⁺-free HEPES-buffered saline solution (HBSS) containing the irreversible SERCA pump inhibitor, thapsigargin (1mM; 15mins), to empty ER calcium stores and prime the nSOCE apparatus. nSOCE was then induced by superfusing with HBSS containing Ca²⁺ (2mM; 15 mins). Somatic calcium responses (F/F₀) of individual cells were recorded and measured using the calcium imaging software, Winfluor (John Dempster, Strathclyde University). Percentage peak nSOCE calcium responses (relative to baseline) in untreated (control) neurons were compared to those evoked in the presence of either the microtubule depolymeriser colchicine (10mM) or the microtubule stabilizer, paclitaxel (10mM). Statistical analyses were conducted using one way ANOVA with Dunnett's multiple comparisons test, with values of $P < 0.05$ considered statistically significant. All data are expressed as mean $\pm$ S.D.

Activation of nSOCE increased intracellular calcium levels above basal levels by $10.3 \pm 11.7\%$, $n = 243$) in untreated neurons. Colchicine (10mM) did not significantly modify peak nSOCE responses relative to control, inducing a peak nSOCE amplitude of $9.7 \pm 6.1\%$ ($n = 97$; $P = 0.99$) above baseline. However, in the presence of the microtubule stabiliser, paclitaxel (10mM), peak nSOCE amplitude was significantly reduced to $6.1 \pm 4.0\%$ ($n=62$; 40.7% reduction relative to control; $P = 0.03$) of control nSOCE responses.

The preliminary data presented here suggest that microtubule stabilisation exerts a significant inhibitory effect on nSOCE activation, possibly by inhibiting the dynamic microtubule reconfiguration required to initiate nSOCE. It is unclear however, why microtubule depolymerisation with colchicine did not exert a similar inhibitory effect on nSOCE.

C32

A case study comparing the physiological responses to graded exercise between a trained runner and a team sport athlete

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Running performance is influenced by multiple physiological determinants, including rate of oxygen uptake ($\dot{V}O_2$), blood lactate concentration and running economy. Physiological testing of team sport athletes has mainly been conducted using field-based assessments, with few laboratory-based studies. This study aims to compare the physiological characteristics of a trained runner (TR) and a team sport athlete (TS) during laboratory-based testing.

Testing was performed in standard (non-plated) running shoes. Following ethical approval and informed consent, two healthy male participants (TR: 39 years, 64.9 kg, 174.9 cm; TS: 19 years, 80.7 kg, 168.2 cm) completed a submaximal graded exercise test (GXT) consisting of 4-minute treadmill running stages beginning ~5 km/h below 10 km race pace and increasing 1 km/h per stage. Following ~5 minutes of recovery during which participants remained ambulatory, a maximal incremental test was performed, initiated with 2 minutes below lactate threshold (LT) 2 (LT2) followed by 1 km/h increases every 30-seconds, until volitional exhaustion. Gas exchange data and heart rate were collected continuously, and RPE (Borg 6-20 scale) was collected in the final 30 seconds of each stage during the GXT. Blood lactate was collected (earlobe) at the end of each 4-minute stage and 2 minutes and 5 minutes post-maximal test. LT1 and LT2 were determined using recognised methods for identifying lactate thresholds during incremental exercise (1, 2). Running economy was calculated as oxygen cost per kilometre at LT1 and LT2.

Physiological profiles for both participants are shown in Table 1. The TR demonstrated a 25.7% higher relative $\dot{V}O_{2\max}$ and a higher $\dot{V}O_{2\max}$ than TS. In contrast, the TS exhibited a lower oxygen cost of running at both LT1 and LT2. Lactate and ventilatory thresholds occurred at higher running speeds in the TR. Similarly, lactate and ventilatory thresholds occurred at a greater percentage of $\dot{V}O_{2\max}$ in the TR, indicating an ability to sustain a higher fraction of their maximum aerobic capacity. Ventilatory thresholds generally corresponded with the lactate thresholds, although a greater discrepancy between LT2 and VT2 was observed in the TR. Both participants demonstrated a plateau in $\dot{V}O_2$ during the final stage of the maximal test, achieved a heart rate within 5% of their age-predicted maximum, reported an RPE ≥ 18 , and achieved an RER > 1.1 .

The TR had higher $\dot{V}O_{2\max}$, $\dot{V}O_{2\max}$ and lactate and ventilatory thresholds occurred at higher relative intensities. However, the TS demonstrated superior running economy, maintaining a lower relative O₂ cost across LT1 and LT2. This novel finding may reflect the physiological adaptations associated with team sports and highlights that superior aerobic capacity does not necessarily coincide with superior running economy. This study provides laboratory-based physiological profiling of a TS athlete, contributing to an area where such research is relatively limited. Multiple physiological determinants rather than a single metric should be evaluated to provide a more comprehensive assessment of an athlete's aerobic performance abilities.

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Variable	Team Sport Athlete	Trained Runner
Relative $\dot{V}O_2$ max (ml/kg/min)	47.9	60.2
Running Economy at LT1 (ml/kg/km)	181.9	199.5
Running Economy at LT2 (ml/kg/km)	187.4	200.5
Velocity at LT1 (km/h)	9.5	16.0
Velocity at LT2 (km/h)	11.3	17.2
Velocity at VT1 (km/h)	9.0	15.0
Velocity at VT2 (km/h)	11.0	16.0
% $\dot{V}O_2$ max at LT1	60.2	88.4
% $\dot{V}O_2$ max at LT2	73.5	95.4
% $\dot{V}O_2$ max at LT1	57.4	84.9
% $\dot{V}O_2$ max at LT2	71.7	88.4

C33

Investigating the Role of A2BAR in Promoting Intestinal Epithelial Wound Healing

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Background: Intestinal epithelial restitution is a rapid, highly coordinated response required to restore barrier integrity after injury. Although the early phases of wound closure rely on migration, cytoskeletal remodelling, and matrix interaction, the upstream signalling pathways that initiate and sustain restitution remain incompletely defined. Adenosine signalling through the A2B adenosine receptor (A2BAR) is elevated during inflammation and hypoxia, suggesting a potential role in epithelial repair, but its mechanistic contribution to wound closure has not been fully characterised. We investigated A2BAR-dependent restitution using human intestinal epithelial monolayers and a physiologically relevant organoid injury model.

Methods: T84 monolayers were scratch-wounded and treated with the selective A2BAR agonist BAY 60-6583 (1 μ M). Wound closure was quantified over 24 h (n = 6 independent experiments). Proliferation was assessed by EdU incorporation (n = 4). Migration was analysed using 12 h time-lapse imaging (n = 4). RNA-seq was performed on wounded monolayers $\pm$ BAY 60-6583 at 6 h (n = 3 biological replicates per condition; FDR < 0.05). Matrix-remodelling genes (MMP1, MMP7, MMP10) were validated by qPCR (n = 5) and Western blotting (n = 3 independent lysates). Murine ileal organoids were generated from C57BL/6 mice (n = 4 mice; HPRA licence compliant). A single-cell laser ablation assay was developed using targeted 405 nm injury, with restitution quantified by live imaging (n = 12 organoids per condition).

Results: BAY 60-6583 significantly accelerated wound closure compared with vehicle ($62.3 \pm 4.1\%$ vs $41.7 \pm 3.8\%$ closure at 24 h; $p < 0.01$, two-tailed t-test). EdU incorporation increased by 1.8-fold ($p < 0.05$), indicating enhanced S-phase entry. Migration velocity increased from 0.42 ± 0.05 μ m/min to 0.67 ± 0.06 μ m/min ($p < 0.01$). RNA-seq identified 266 differentially expressed genes enriched for Neuregulin signalling, epithelial migration, and wound-healing pathways. Upstream regulator analysis predicted activation of ELK1, MYC, and STAT5. A focused seven-gene signature linked A2BAR signalling to migration-associated repair. MMP1 was the most strongly induced transcript (11.4-fold; FDR < 0.001), consistent with its elevated expression in ulcerative colitis and Crohn's disease datasets. BAY 60-6583 increased MMP1 (6.2-fold), MMP10 (3.9-fold), and context-dependent MMP7 (2.1-fold) expression by qPCR ($p < 0.05$ for all). Western blots (n = 3) confirmed increased MMP1 protein. Mechanistic interrogation suggested modulation of AP-1/FOS family transcription factors rather than a purely cAMP/PKA-dependent pathway, supported by increased FOSL1 and JUNB expression (RNA-seq and qPCR; $p < 0.05$). Murine ileal organoids expressed canonical epithelial lineage markers (Lgr5, Muc2, ChgA) and matrix-remodelling genes. Single-cell laser ablation produced immediate membrane blebbing and rapid neighbouring cell movement into the damaged site. Restitution occurred within 8.3 ± 1.1 min (n = 12), establishing a tractable ex vivo platform for quantifying epithelial repair.

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Conclusion: A2BAR activation promotes epithelial restitution by enhancing proliferation, migration, and AP-1-dependent matrix remodelling, with MMP1 emerging as a key downstream effector. The organoid laser-wounding assay provides a physiologically relevant system for future mechanistic and therapeutic studies.

C34

Defining the glioma peritumoural zone: A multi-disciplinary Delphi consensus establishing the Radiology-Pathology-Distance (RPD) Classification

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Objective

To establish an international, multidisciplinary consensus definition and classification framework for the glioma peritumoural zone (PTZ), applicable to research and clinical practice.

Design

International, multidisciplinary modified Delphi study.

Subjects

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Multidisciplinary experts (neurosurgery, neuroradiology, neuropathology, neuro-oncology and pre-clinical science) from 23 countries and 10 professional societies completed two survey rounds (round 1, n=61; round 2, n=80). Distributing societies comprised: European Association of Neurosurgical Societies (EANS), European Society of Neuroradiology (ESNR), European Confederation of Neuropathological Societies (Euro-CNS), Deutsche Gesellschaft für Neurochirurgie (DGNC), Société Française de Neurochirurgie (SFNC), Société Française de Neuroradiologie (SFNR), British Neuro-Oncology Society (BNOS), British Neuropathological Society (BNS), Irish Neuropathology Society (IPNS) and the Society of British Neurological Surgeons (SBNS).

Method

A two-round modified Delphi process was conducted with predefined consensus threshold of $\geq 70\%$ agreement. Participants were recruited via literature review and distribution via 10 collaborating neuro-oncology societies. Iterative survey rounds refined PTZ definitions and informed development of a unified classification framework.

Results

Consensus supported a three-axis Radiology–Pathology–Distance (RPD) classification. The PTZ was defined radiologically as the zone beyond tumour mass (encompassing both contrast-enhancing and non-contrast enhancing components) on structural MRI (81.81%), pathologically as a transitional zone of variably infiltrated but largely preserved brain parenchyma implicated in tumour infiltration and/or microenvironmental change (84.31%), and spatially by standardized measurement of sampling distance (71.64%). The RPD framework supports integration of annotated datasets through a simple alphanumeric coding framework.

Conclusion

The consensus RPD framework is a clinically-aligned, multi-modal classification system that supports standardised reporting of the glioma peritumoural zone for research and clinical practice.

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Dynamic cerebral autoregulation is unaffected by acute high intensity interval exercise in inactive middle-aged adults

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Background and Purpose The impact of high intensity interval exercise (HIIE) on cerebrovascular function continues to gain interest, particularly surrounding the safety of such exercise in at-risk and ageing populations. HIIE is characterised by intermittent increases in hemodynamic and ventilatory responses during exercise, and may pose a substantial challenge to dynamic cerebral autoregulation (dCA) in the acute post-exercise recovery period. Furthermore, whether the intensity of an acute bout of HIIE impacts dCA recovery post-exercise has not been investigated. Therefore, this study aimed to investigate the acute effect of two popular, time-matched HIIE protocols on dCA in inactive, middle-aged adults.

Methods Eleven inactive middle-aged adults (5 females, mean±SD age: 53.8±6.8 years, BMI: 27.5±4.3kg/m², maximal oxygen uptake (VO₂max): 29.9±5.4 ml/kg/min) participated in this study, which involved four visits. Participants completed a ramp incremental and verification test to exhaustion on a cycle ergometer in the first visit, to determine VO₂max, the gas exchange threshold (GET) and the difference between VO₂max and GET (Δ). All participants then completed three time-matched visits in a randomised and counterbalanced order: 1) a 30min seated control condition (CON), 2) a 30-min HIIE protocol involving 10x 1 min intervals at 70% Δ interspersed with 1 min recovery (HIIE10), and 3) a 30-min HIIE protocol involving 4x 4 min intervals at 20% Δ interspersed with 3 min recovery (HIIE4). dCA was assessed before, 10 min and 60 min after each condition using 5 min of repeated sit-stands performed at a frequency of 0.05 Hz. Transcranial Doppler ultrasound continuously measured middle cerebral artery blood velocity (MCAv), and beat-to-beat blood pressure was measured using finger plethysmography. dCA was analysed using transfer function analysis of MCAv and mean arterial pressure, with the effect of time and condition on dCA explored using two-way repeated measures ANOVAs.

Results There was no significant main effect of time, condition or interaction effect for dCA gain ($P \geq 0.21$, $\eta^2 \leq 0.14$) or normalised gain ($P \geq 0.40$, $\eta^2 \leq 0.09$). Furthermore, dCA coherence and phase also did not differ across condition ($P \geq 0.48$, $\eta^2 \leq 0.07$) or time ($P \geq 0.27$, $\eta^2 \leq 0.12$), and there were no significant condition*time interactions ($P \geq 0.11$, $\eta^2 \leq 0.19$).

Conclusions These findings indicate that the ability of the cerebrovasculature to buffer forced oscillations in blood pressure are maintained following two different types of HIIE in inactive middle-aged adults. dCA recovery during the one hour following exercise was not impacted by the intensity of the HIIE bout, and suggests that, from a cerebral autoregulatory perspective, these bouts are safely tolerated in this population.